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Mesenchymal Stem Cell-Based Therapy for Prostate Cancer

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| 14. ABSTRACT Although the rate of advances in prostate cancer research is rapidly accelerating, there still remains an urgent need for development of more effective therapy for castrate resistant metastatic prostate cancer (CRPC). Based upon a substantial published literature from multiple groups, as well as unpublished studies to be presented from the applicants laboratories, an exciting approach that has not been tested clinically involves isolating a specific type of healthy bone marrow derived cells and loading it with a therapeutic chemical so that when these loaded cells are injected into the blood stream, they are selectively retained (i.e., "home") to metastatic sites of cancer in castration resistant metastatic prostate cancer patients. The therapeutic chemical delivered via these injected cells is selectively engineered to act like a "molecular grenade" in that it is designed to "detonate" upon release of a non-selective toxin restrictively within the microenvironment of metastatic sites of cancer. This approach is both exciting and practical because the cells used for this selective cancer delivery of the molecular grenade can be routinely harvested from healthy bone marrow donors and do not need to be host matched and have been safely infused into humans to treat other non-cancer diseases. To develop such a cell based molecular grenade delivery as systemic therapy for metastatic CRPC, a multi-disciplinary/multi-institutional/multi-investigator team has been assembled based upon the synergistic (i.e., team) expertise needed to translating the basic science discoveries concerning cancer homing into clinical trials for CRPC. | | | | | |
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Introduction

Although the rate of advances in prostate cancer research is rapidly accelerating, there still remains an urgent need for development of more effective therapy for castrate resistant metastatic prostate cancer (CRPC). One exciting approach that has never been tested clinically involves the use of cellular therapy to deliver toxic payloads to sites of prostate cancer. The rationale for this approach is based upon the substantial data in pre-clinical models documenting bone marrow derived Mesenchymal Stem Cells (MSCs) are mobilized into circulation in response to inflammatory stimuli due to the expression of an extensive array of chemokine receptors on their surface. Once in the blood stream, MSCs home to sites of inflammation, including those present at sites of primary and metastatic prostatic cancer, as a result of the chemokine gradient originating from these lesions. MSCs are defined by the co-expression of CD90, CD73, and CD105 in the absence of hematopoietic lineage markers, including CD14, CD20, CD34, CD45, and HLA-DR.

These results are exciting because allogeneic hbMSCs are non-immunogenic and can be routinely harvested from healthy bone marrow donors and expanded *ex vivo* using Federal Drug Administration (FDA)-approved protocols. Due to their lack of immunogenicity, these allogeneic hbMSC do not need to be host matched and thus have been infused to treat graft versus host disease, inflammatory bowel disease and myocardial infarction in clinical trials. These clinical studies have documented that hundreds of millions of allogeneic hbMSCs can be safely given IV without significant side effects. To date, however, no trial has evaluated IV infused allogeneic hbMSCs in any cancer patient, including those with prostate cancer.

Body

The **hypothesis** of this program is that non-virally modified allogeneic hbMSCs can be isolated and then load *in vitro* with a therapeutic chemical so that when these loaded cells are injected into the blood stream, they are selectively retained (i.e., “home”) to metastatic sites of cancer in castration resistant metastatic prostate cancer patients. The therapeutic chemical delivered via these injected cells is selectively engineered to act like a “molecular grenade” in that it is designed to “detonate” upon release of a non-selective toxin restrictively within the microenvironment of metastatic sites of cancer thus sparing host toxicity. This approach is both exciting and practical because the hbMSCs used for this selective cancer delivery of the molecular grenade can be routinely harvested from healthy bone marrow donors and do not need to be host matched and have been safely infused into humans to treat other non-cancer diseases.

To develop such “Trojan Horse” MSCs as systemic therapy for metastatic CRPC, a multi-disciplinary/multi-institutional/multi-investigator synergistic team was assembled with the common goal of translating the basic science discoveries concerning hbMSCs cancer homing ability into clinical trials for CRPC. The team is composed of Drs. Jeffrey Karp Co-Director of Regenerative Therapeutics at the Brigham & Women's Hospital, Harvard Medical School, Samuel Denmeade and John Isaacs of the Chemical Therapeutic Program of the Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins. Each of these individuals brings a unique-world class expertise to this collaborative team effort.

Key Research Accomplishments:

Identification of MSCs in the Developing Human Prostate

A rapid tissue dissociation protocol and multi-parameter flow cytometry assay was optimized for the quantification of MSCs from prostate tissue (1). To identify MSCs in human UGS, a multi-parameter flow cytometry assay based on the co-expression of CD73, CD90, and CD105 in the absence of CD14, CD20, CD34, CD45, and HLA-DR expression was used. Based on this analysis, MSCs represent 2.86% (range 1.84-4.43%) of the cells present in 15-20wk old human UGS (**Table 1**). This

is significantly enriched compared to an age-matched 17wk old umbilical cord in which 0.9% of the cells were defined as MSCs (data not shown). Consistent with the high replicative potential associated with a stem cell phenotype, rapid enrichment of the MSC population was observed following the expansion of UGM-derived stromal cells in tissue culture. In all seven independent cultures analyzed, the majority of cells were consistent with an MSC phenotype within just a few (≤ 3) passages (**Table 2**).

| Sample | Age (wks) | % MSCs |
|--------|-----------|--------|
| UGS-1 | 15 | 2.24 |
| UGS-2 | 16 | 4.43 |
| UGS-3 | 17 | 1.97 |
| UGS-4 | 17 | 3.81 |
| UGS-5 | 20 | 1.84 |

Table 1: Percent of cells derived from fetal urogenital sinus (UGS) defined as MSCs.

| Sample | % MSCs |
|--------|--------|
| UGS-c1 | 89.5 |
| UGS-c2 | 56.2 |
| UGS-c3 | 86.2 |
| UGS-c4 | 89.5 |
| UGS-c5 | 95.6 |
| UGS-c6 | 96.0 |
| UGS-c7 | 85.2 |

Table 2: Percent of cells defined as MSCs in cultures derived from fetal urogenital sinus (UGS) at passage 3.

Identification of MSCs in Healthy, Young Adult Prostates

To determine whether MSCs are only present in the fetal prostate during organogenesis or persist into adulthood, we examined prostates obtained from young adults (<25 yrs old) through a rapid organ donor program. Again, a subset of cells in all of the samples analyzed were consistent with an MSC phenotype (**Table 3**). As expected, MSCs represent a relatively low percentage of the overall cells present within the prostate (range: 0.03 – 3.15%). However, >3% of the cells present in one case were MSCs, a significantly higher number than observed in the majority of cases. Histological examination of the tissue revealed evidence of a mild chronic inflammatory infiltrate (**Table 3**). In contrast, inflammation was not observed in the other samples within this age range.

| Sample | Age (yrs) | Tissue Source | % MSCs | Inflammation |
|---------|-----------|---------------|--------|--------------|
| nPrSC-1 | 17 | Autopsy | 0.19 | None |
| nPrSC-2 | 21 | Organ Donor | 0.03 | None |
| nPrSC-3 | 21 | Organ Donor | 0.58 | None |
| nPrSC-4 | 24 | Organ Donor | 3.15 | Mild |

Table 3: Percent of cells defined as MSCs in prostate tissue from healthy donors <25 yrs of age. This sample was used as representative of 'normal' tissue from an older individual. Samples were scored by a pathologist for the type (chronic or acute) and intensity (none, mild, moderate, or severe) of inflammation present. Unless otherwise noted, inflammation was scored as chronic.

Identification of MSCs in Benign Prostatic Hyperplasia (BPH)

Next, the presence of MSCs in pathological tissue from older men was investigated. Using the same multi-parameter flow cytometry method, MSCs were found to represent from below the level of detection (<0.01%) to 2.31% of all cells present in prostate tissue derived from men undergoing an open prostatectomy for symptomatic BPH (**Table 4**). Again, the majority of cases have a fairly low frequency of MSCs present with the exception of a single case that has >2%, which also had

histologic evidence of mild chronic inflammation. In contrast to the normal tissue, other samples within this cohort also had evidence of inflammation including one with a more intense infiltrate (moderate).

| Sample | Age (yrs) | Surgical Procedure | % MSCs | Inflammation |
|--------|-----------|--------------------|--------|--------------|
| BPH-1 | 64 | Open | 0.04 | Moderate |
| BPH-2 | 76 | Open | 0.26 | None |
| BPH-3 | 83 | Open | 2.31 | Mild |
| BPH-4 | | Open | < 0.01 | Mild |
| BPH-5 | | Open | 0.08 | |

Table 4: Percent of cells defined as MSCs in primary BPH tissue obtained from open prostatectomies. Samples were scored by a pathologist for the type (chronic or acute) and intensity (none, mild, moderate, or severe) of inflammation present. Unless otherwise noted, inflammation was scored as chronic.

Identification of MSCs in Prostate Cancer

We previously reported that MSCs represent from <0.1% to 1.10% of the cells present in prostate tissue (n = 10) from men undergoing radical prostatectomy for prostate cancer (Brennen et al. Oncotarget, 2013). The current study extends those observations into >30 cases (**Table 5**). Consistent with our previous observations, there does not appear to be any correlation with Gleason score in this series (**Fig. 1a**). However, in contrast to the expected Gaussian distribution, a bimodal distribution pattern is observed with the majority of cases having <0.5% and a subset of cases (12%) enriched in MSCs (~1%) (**Fig.1b**). Of note, only 1 of 10 high grade cancers (Gleason ≥ 8) in this series were in this enriched MSC fraction.

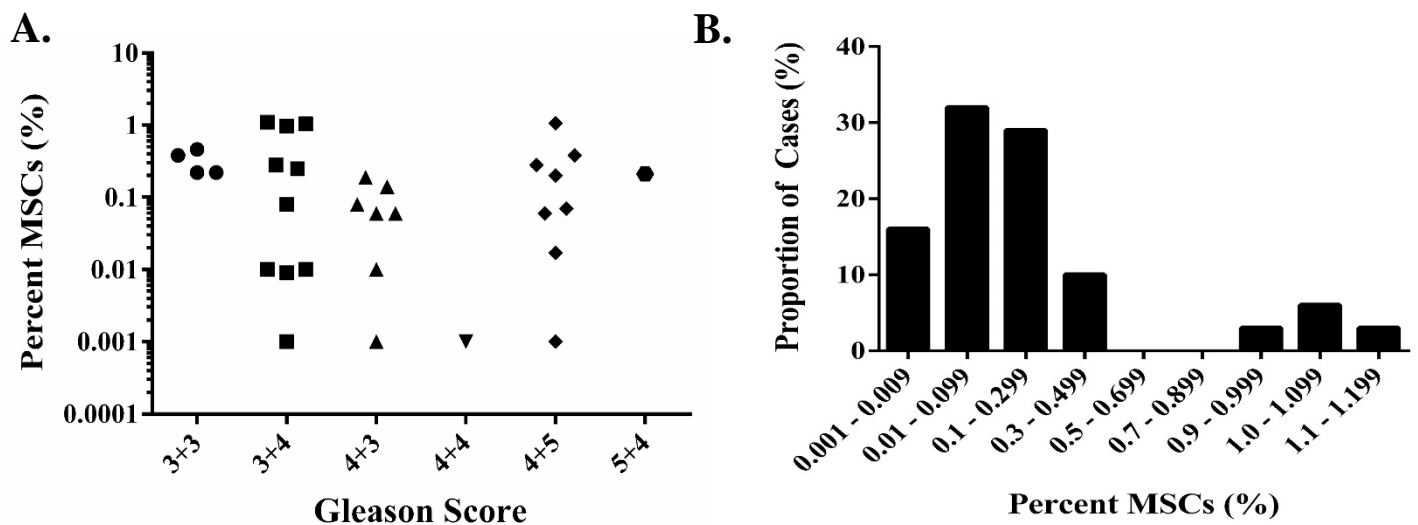


Figure 1: Distribution of MSC frequency and correlation with Gleason Score. (A) No correlation is observed between the number of MSCs detected in radical prostatectomy tissue and Gleason score. (B) A bimodal distribution in the percentage of MSCs detected in radical prostatectomy tissue is observed with the majority of cases (88%) having <0.5% MSCs and subset (12%) enriched in MSCs with ~1% of all cells defined as MSCs.

Association between Infiltrating MSCs and Inflammation in Malignant Prostates

To determine whether the number of MSCs detected in prostatectomy samples was simply a function of the level of inflammation present in the tissue, two pathologists performed a blinded

analysis of the specimens. Prostatectomy tissue was scored for the type of inflammation (acute or chronic), the level of intensity (mild, moderate, severe), and whether it was focal or widespread in benign, atrophic and malignant areas. No association between MSCs and inflammation was detected (**Table 5**). Inflammation was more frequently observed in benign areas of malignant prostates; though it was commonly detected in benign, atrophic, and malignant areas (65%, 45%, and 43%, respectively). Additionally, the inflammation observed was typically of the chronic, focal and relatively mild.

| Tissue Type | Sample | Age (yrs) | % MSC | Gleason Score | Inflammation | | |
|-------------|----------|-----------|--------|---------------|--------------|----------------|----------|
| | | | | | Cancer | Benign | Atrophy |
| Cancer | PrCSC-1 | 69 | 1.10 | 3+4 | Mild | None | Moderate |
| | PrCSC-2 | 70 | 1.06 | 4+5 | None | Mild | - |
| | PrCSC-3 | 66 | 1.03 | 3+4 | - | Moderate | - |
| | PrCSC-4 | 55 | 0.98 | 3+4 | None | Mild | - |
| | PrCSC-5 | 60 | 0.45 | 3+3 | - | Mild | Mild |
| | PrCSC-6 | 70 | 0.38 | 3+3 | Mild | Mild | - |
| | PrCSC-7 | 65 | 0.38 | 4+5 | N/A | N/A | N/A |
| | PrCSC-8 | 63 | 0.28 | 4+5 | None | Mild | None |
| | PrCSC-9 | 68 | 0.28 | 3+4 | None | Mild | Mild |
| | PrCSC-10 | 70 | 0.25 | 3+4 | Mild | Mild | None |
| | PrCSC-11 | 59 | 0.23 | 4+5 | Mild | Mild | Mild |
| | PrCSC-12 | 51 | 0.22 | 3+3 | None | Mild | - |
| | PrCSC-13 | 58 | 0.22 | 3+3 | - | Acute Mild | None |
| | PrCSC-14 | 70 | 0.21 | 5+4 | - | None | None |
| | PrCSC-15 | 55 | 0.19 | 4+3 | Mild | Moderate | Mild |
| | PrCSC-16 | 65 | 0.14 | 4+3 | None | None | None |
| | PrCSC-17 | 61 | 0.08 | 3+4 | Mild | None | None |
| | PrCSC-18 | 70 | 0.08 | 4+3 | Mild | Mild | None |
| | PrCSC-19 | 57 | 0.07 | 4+5 | - | Acute Moderate | - |
| | PrCSC-20 | 54 | 0.06 | 4+3 | None | None | None |
| | PrCSC-21 | 59 | 0.06 | 4+3 | - | None | - |
| | PrCSC-22 | 59 | 0.06 | 4+5 | Mild | Mild | Mild |
| | PrCSC-23 | 64 | 0.06 | 4+3 | None | None | None |
| | PrCSC-24 | 64 | 0.06 | 4+3 | None | None | None |
| | PrCSC-25 | 59 | 0.017 | 4+5 | - | None | Mild |
| | PrCSC-26 | 57 | 0.01 | 3+4 | Mild | None | - |
| | PrCSC-27 | 73 | 0.01 | 3+4 | - | None | Mild |
| | PrCSC-28 | 70 | 0.009 | 3+4 | None | None | None |
| | PrCSC-29 | 63 | <0.01 | 4+3 | Moderate | Mild | None |
| | PrCSC-30 | 49 | <0.001 | 4+4 | None | None | - |
| | PrCSC-31 | 53 | <0.001 | 4+3 | None | Mild | - |
| PrCSC-32 | 62 | <0.001 | 4+5 | None | Mild | - | |
| PrCSC-33 | 68 | <0.001 | 3+4 | - | Mild | Mild | |

Table 5: Percent of cells defined as MSCs in radical prostatectomy tissue from men with prostate cancer. No association between the number of MSCs detected and the level of inflammation present in the tissue was observed. Benign, atrophic, and malignant areas were scored where appropriate for the type (chronic or acute) and intensity (none, mild, moderate, or severe) of inflammation present. Unless otherwise noted, all inflammation observed was chronic. No tissue was available for IHC analysis in PrCSC-7. PrCSC-18 had areas of chronic and acute inflammation.

Functional Analysis of Multipotent Differentiation Potential

In addition to the analytical strategy just described, a functional assay was performed to confirm the multipotent differentiation potential of these different prostate-derived MSCs. In contrast to the bone marrow-derived MSCs, those derived from fetal and adult prostates are unable to generate adipocytes, only chondrocytes and osteoblasts (**Table 6**). Evidence from the literature suggests that androgen signaling suppresses adipocyte differentiation in MSCs (25, 26). Consistent with this observation, fetal prostate stromal cells and those from healthy adult prostates express the androgen receptor (AR) (**Fig. 2**). In contrast, bone marrow-derived MSCs only express very low levels of AR. Interestingly, a subset of stromal cultures derived from men with prostate cancer also have low levels of AR expression (**Fig. 2**) and retain the ability to differentiate along the adipocyte lineage (**Table 6**). However, incubation of bone marrow-derived MSCs with dihydrotestosterone (DHT, 30 nM) was unable to suppress adipocyte differentiation nor did culturing prostate-derived MSCs in the presence of bicalutamide (300 nM) restore this potential (data not shown).

| Sample | Adipocyte | Osteoblast | Chondrocyte |
|-----------|-----------|------------|-------------|
| BM-MSC-c1 | + | + | + |
| BM-MSC-c2 | + | + | + |
| BM-MSC-c3 | + | + | |
| BM-MSC-c4 | + | + | |
| PrCSC-c32 | + | + | - |
| PrCSC-c33 | + | + | + |
| PrCSC-c34 | + | - | - |
| PrCSC-c35 | + | + | + |
| PrCSC-c36 | - | - | - |
| PrCSC-c37 | + | + | + |
| PrCSC-c38 | - | + | |
| PrCSC-c39 | - | + | |
| PrCSC-c40 | - | + | |
| BPH-c2 | - | + | +/- |
| BPH-c4 | - | + | |
| nPrSC-c1 | - | + | |
| nPrSC-c4 | - | + | |
| nPrSC-c5 | - | + | |
| nPrSC-c6 | +/- | + | +/- |
| nPrSC-c7 | - | + | + |
| nPrSC-c8 | - | + | + |
| nPrSC-c9 | - | + | + |
| nPrSC-c10 | - | + | |

| | | | |
|-----------|---|---|---|
| nPrSC-c11 | - | + | + |
| nPrSC-c12 | | | + |
| UGS-c1 | - | + | + |
| UGS-c2 | - | + | + |
| UGS-c3 | - | + | + |
| UGS-c4 | - | + | + |
| UGS-c5 | - | + | |
| UGS-c6 | | + | |
| UGS-c7 | - | + | |

Table 6: Multipotent differentiation potential of MSCs from various sources. In contrast to bone marrow-derived MSCs, those obtained from fetal, normal or BPH tissue are unable to differentiate into adipocytes. However, a subset of prostate cancer specimens contain MSCs that retain tri-lineage differentiation potential, suggesting the recruitment of more naïve progenitors from the bone marrow.

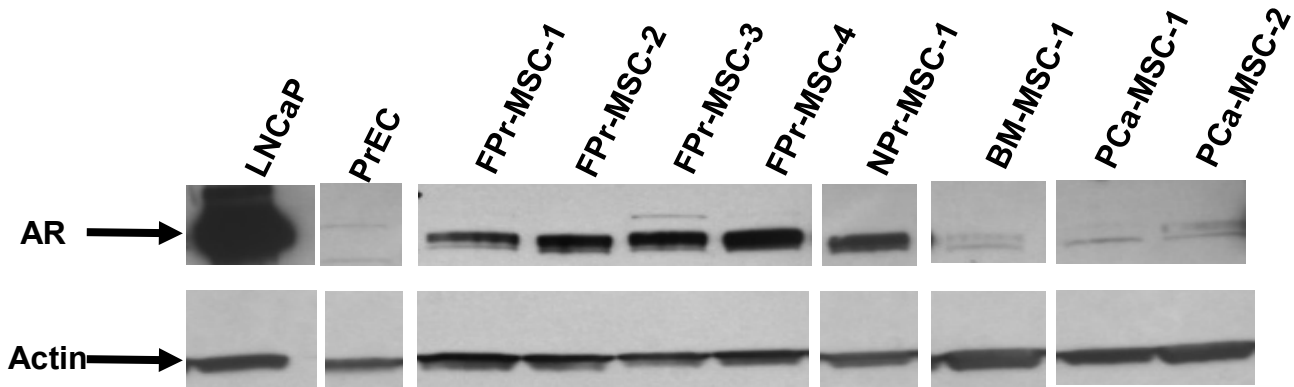


Figure 2: Androgen Receptor (AR) expression in MSCs derived from various sources. MSCs from fetal and normal prostate tissue express higher levels of AR than those obtained from bone marrow and prostate cancer tissue. LNCaP and PrEC were used as positive and negative controls, respectively.

Quantitation of MSC homing to sites of Prostate Cancer

Two complimentary methods have been developed to quantitate the homing ability of allogeneic hbMSCs to sites of prostate cancer for the clinical vs. preclinical xenograft studies. The use of 2 methods is required because in the human trial, the infused hbMSCs must be genetically and chemically unmodified (i.e., unlabeled cells) while for the xenograft studies, these cells can be radioactively (Cr51) labeled. For the human trial, digital PCR is being used to quantify the unlabeled MSC homing to sites of primary prostate cancer, the Isaacs' lab has interacted with Inostics (Hamburg, Germany) and contracted them to use BEAMing (Beads, Emulsion, Amplification, Magnetics) technology on the infused patient prostatectomy specimens. BEAMing is a form of digital PCR, which allows the non-biased amplification of rare alleles for quantitative detection of somatic mutations with high sensitivity and selectivity by creating a water-in-oil emulsion that partitions the sample into millions of individual reactions. A panel of 6 SNPs from stable genomic regions in prostate cancer has been identified that is suitable for differentiating donor allogeneic hbMSCs from recipient cells (i.e., probability of identical profile: 1 in 4,049). This panel was validated using three prostate cancer epithelial cell lines (LNCaP, LAPC-4, and VCaP) and three bone marrow-derived primary hbMSC cultures (ET20085, JDP22885, and PW19023- these are the 3 donors we are using for the Phase 0 trial), which confirms non-overlapping SNP profiles within these samples to permit

donor/host differentiation. Additionally, the compatibility of this technology with primary tissue samples has also been demonstrated using cores from primary prostatectomy specimens comparable to what will be obtained as part of the trial protocol. This panel has been used to generate a “MSC standard curve” for determination of the assay-specific limit of detection.

This “MSC standard curve” consisted of a dilution series of MSCs spiked into suspensions of three different prostate epithelial cell lines (LNCaP, LAPC-4, VCaP). The sensitivity of the assay allows us to detect donor cells representing as few 0.01% of the sample, (**Figure 3**). This sensitivity is in the predicted range for MSCs homing to prostate based on our preliminary data (0.01-1.1%). White blood cells from each of the three allogeneic hbMSC donors have been isolated and shipped for analysis of the SNP profiles

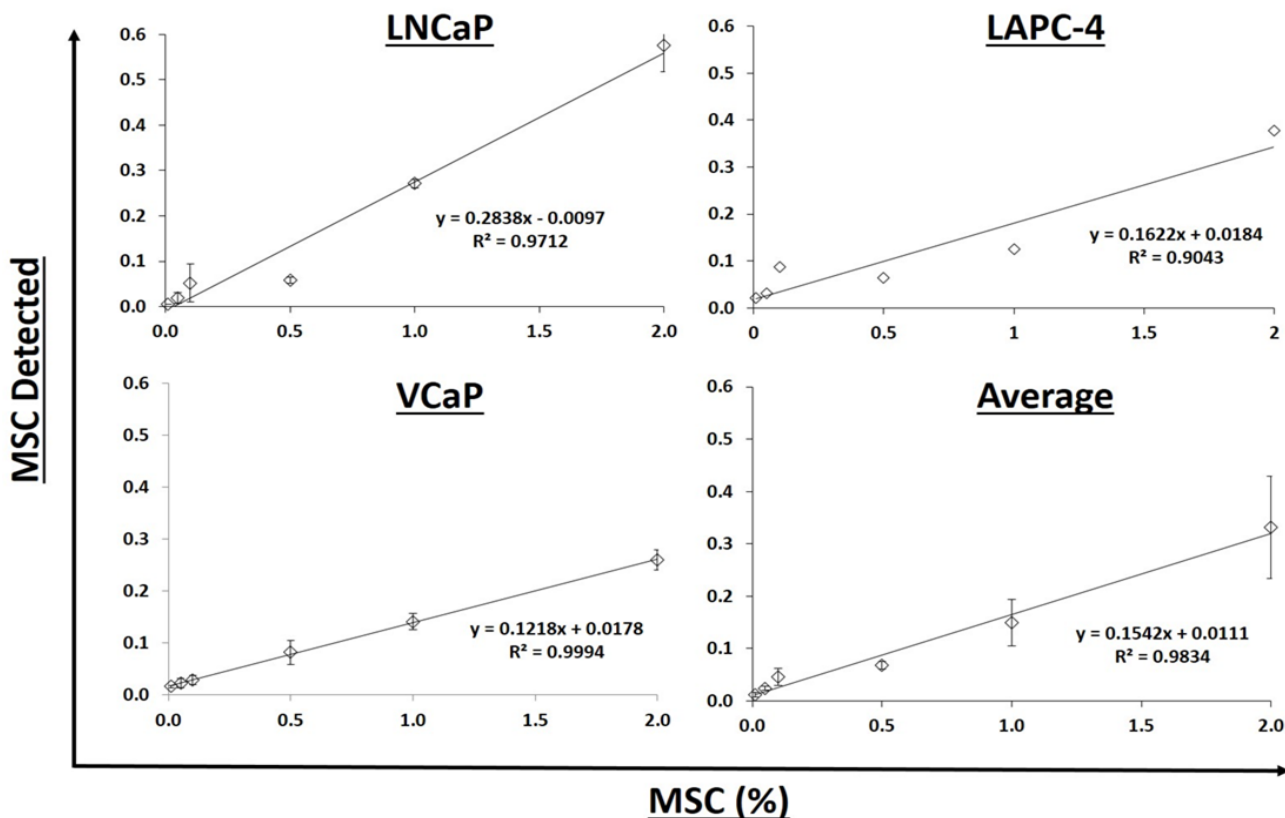


Figure 3: Standard curves of hbMSCs spiked into different prostate cancer cell lines and analyzed using BEAMing-digital PCR

Using this digital PCR method, we are determining the number of hbMSCs which home to sites of prostate cancer following infusion of 2×10^8 in human males. The results are expressed as the number of MSCs homing per gram of tissue. Based upon these results, we will infuse mice bearing human prostate cancer xenografts with varying number of allogeneic hbMSCs to determine the infusion number which produces a similar number of MSC homing to prostate cancer as in patients.

While digital PCR is very accurate and does not require modifying the cells in any way, it is expensive and time consuming. Therefore, in our preclinical xenograft studies, we are not using this method for quantitation of infused allogeneic hbMSCs. Instead, we are using the well-established method of radiolabeling these cells before infusion into prostate cancer bearing nude mice so that the

radioactive associated with any tissue can be used to determine the number of radioactive hbMSCs which have homed to that site. To do this, we have developed and validated a method for incubating hbMSCs with radioactive sodium chromate ($^{51}\text{CrO}_4$). Sodium chromate is cell permeable, but following reduction to trivalent chromium ion intracellularly it becomes impermeable due to crosslinking to macromolecules. Chromium-labeled cells (1×10^6) were then injected IV into animals bearing CWR22 xenografts. Whole tissues were excised at the respective time points and total chromium was measured using a gamma counter, which was used to determine the number of cells present in each tissue (**Fig. 4**). Using this assay, ~ 2 cells/mg or 0.1% of the injected cells were detected in tumor tissue and this remained stable over a one week period, with the majority of these cells trafficking to the lung, liver, kidney, and spleen.

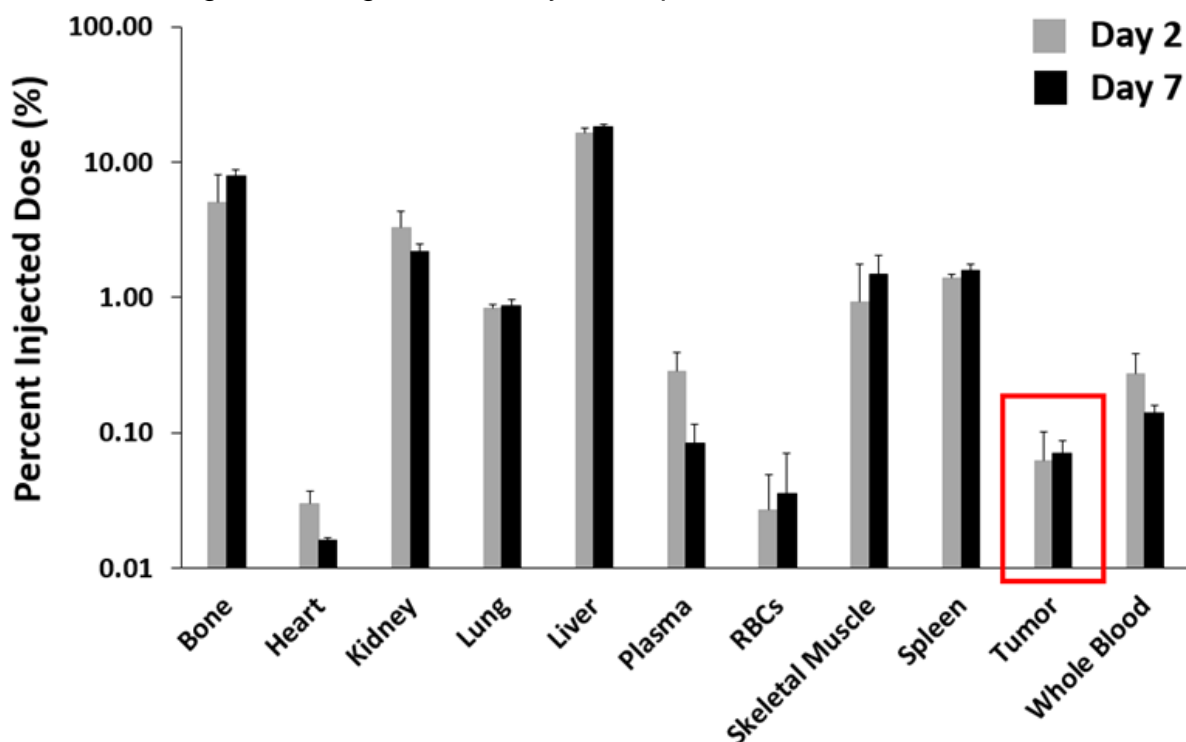
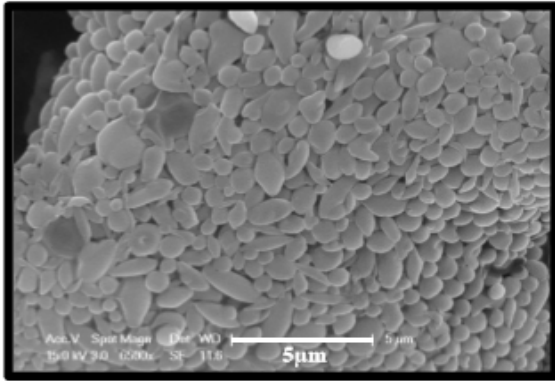


Figure 4: Biodistribution of systemically infused MSCs. Chromium (^{51}Cr)-labeled MSCs (1×10^6) were injected IV into animals bearing CWR22 xenografts. Tissues were excised at the indicated time point and total chromium per tissue was assayed using a gamma counter.

The wide biodistribution and low numbers of MSCs that traffic to tumors following systemic infusion provides further support for the need to use of a highly potent prodrug selectively activated within sites of cancer, such as a PSA-activated prodrug.

Microparticles loaded with a PSA-activated Prodrug

Microparticles encapsulating a PSA-activated thapsigargin-based prodrug (G115, **Fig. 5**) were generated by the Karp lab with the properties outlined in **Table 7**. These microparticles were shown to stable in lyophilized form and release the drug following resuspension in an aqueous buffer (data not shown). Microparticle internalization in MSCs was confirmed using confocal microscopy (**Fig. 5A**), and LCMS demonstrated release of the prodrug from microparticle-loaded MSCs over at least a 1wk period with a very small fraction ($<1\%$) converted to the active form of the drug (**Fig. 6B**).



G115-loaded PLGA MPs

| Property | Mean ± SD |
|------------------------------|---------------|
| Size (nm) | 1127.2 ± 89.5 |
| Polydispersity | 0.366 ± 0.10 |
| Charge (mV) | -21.64 ± 6.11 |
| Drug loading (%) | 13.28 ± 1.40 |
| Encapsulation efficiency (%) | 88.7 ± 5.5 |

Figure 5: SEM images of Microparticles loaded with a PSA-activated prodrug.

Table 7: Properties of G115-loaded

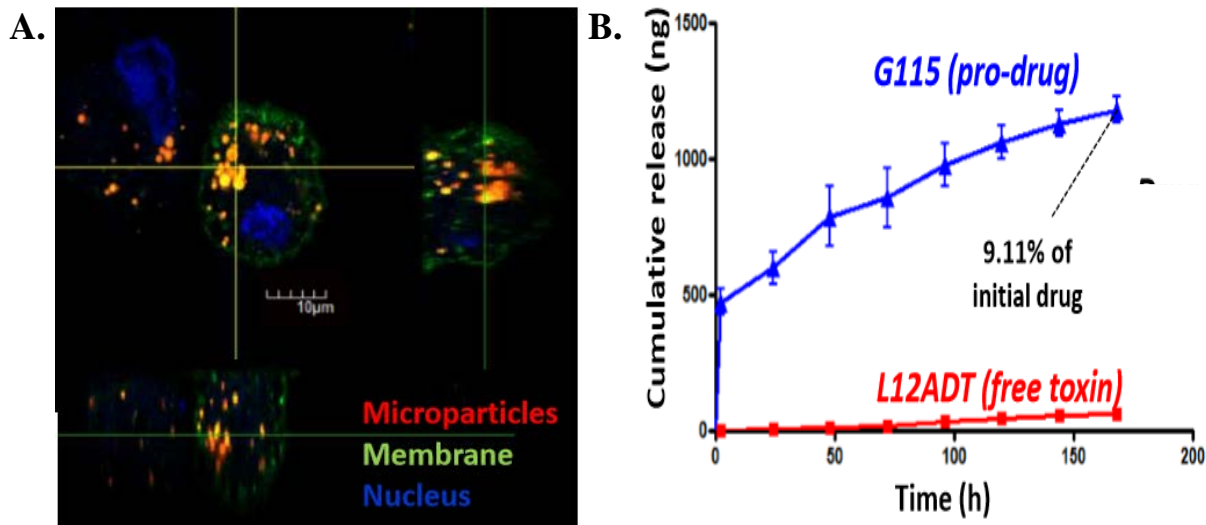


Figure 6: Microparticle internalization and release of prodrug from loaded MSCs. (A) Confocal microscopy demonstrating internalization of microparticles following overnight incubation. Microparticles = green; Membrane = red; Nucleus = blue. (B) Release of prodrug from MSCs loaded with G115 microparticles as determined by LCMS.

The released drug was shown to be toxic to PSA-producing LNCaP cells using either microparticle-conditioned supernatant (**Fig. 6A**) or in a Transwell co-culture assay (**Fig. 6B**).

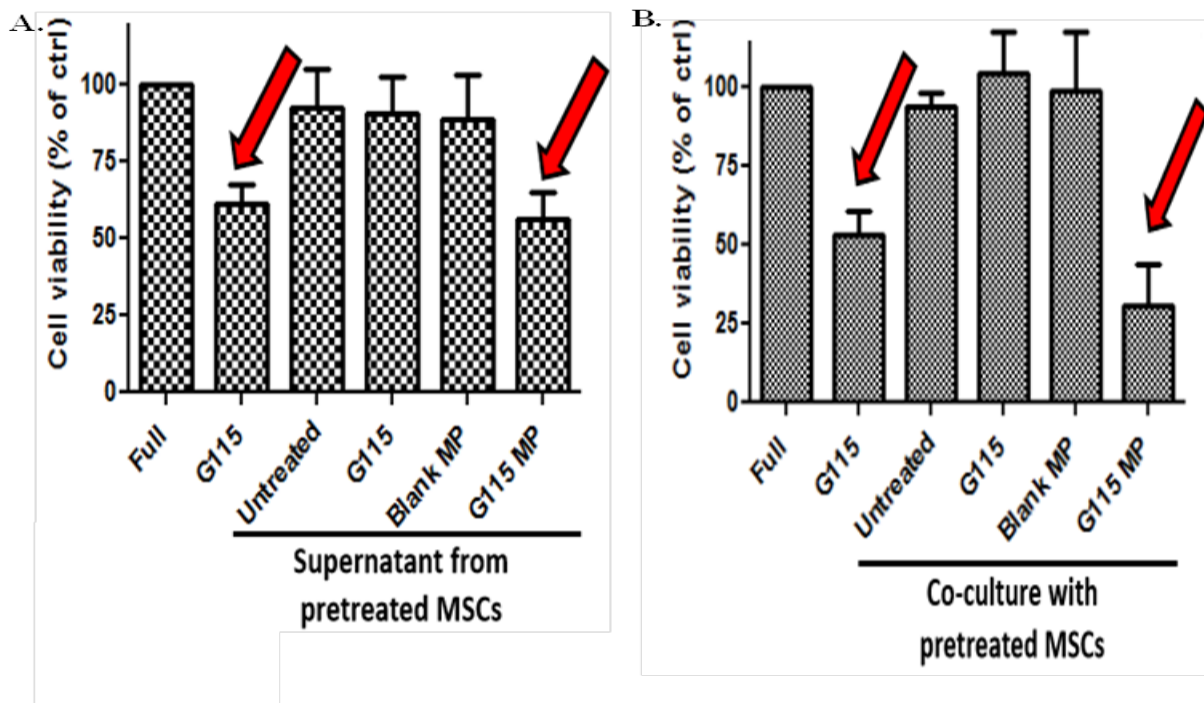


Figure 6: Prodrug released from MSCs loaded with G115 microparticles is toxic to PSA-producing LNCaP cells. (A) G115-loaded microparticles were incubated in buffer, and the supernatant was placed on LNCaP cells. Only the supernatant from MSCs loaded with G115 microparticles had a cytotoxic effect. (B) MSCs loaded with G115 microparticles were seeded into the upper chamber of a Transwell tissue culture plate containing LNCaP cells. Again, only MSCs loaded with G115 microparticles had a cytotoxic effect.

Reportable Outcomes

Patients with >0.9% infiltrating MSCs are not restricted to histologically high grade cancers (i.e., only 1/8 Gleason score 8-10 cancers had >0.9% MSCs). Therefore, the bimodal distribution in the percent of infiltrating MSCs suggests that a cut-off value (e.g., 0.9%) may identify patients with localized cancer at high risk for progression to advanced disease and provide prognostic information independent of Gleason score. This discovery is being followed up by Dr. Nathaniel Brennen (i.e., a newly appointed faculty member at Hopkins who also is a PCF Young Investigator awardee) via a Maryland Cigarette Restitution Fund Grant entitled: "Tumor-infiltrating Mesenchymal Stem Cells (MSCs) as a function of the Patient's Immune Response: potential as a predictive marker of prostate cancer aggressiveness in the African American population."

These results are exciting because allogeneic hbMSCs are non-immunogenic and can be routinely harvested from healthy bone marrow donors and expanded ex vivo using Federal Drug Administration (FDA)-approved protocols. Due to their lack of immunogenicity, these allogeneic hbMSC do not need to be host matched and thus have been infused to treat graft versus host disease, inflammatory bowel disease and myocardial infarction in clinical trials.

Conclusions

These results document two things. **First**, the therapeutic agent loaded into hbMSCs must be a prodrug which is selectively activated only by prostate cancer cells so that it is not toxic to lung, liver, kidney, or spleen cells and **second**, the toxin selectively liberated from the prodrug by prostate cancer cells must be highly toxic within the prostate cancer microenvironment since only a small amount of prodrug can be delivered to the sites of prostate cancer.

References:

1. Brennen WN, Chen S, Denmeade SR, Isaacs JT. Quantitation of mesenchymal stem cells (MSCs) at sites of human prostate cancer. *Oncotarget* 2013; 4: 106-17.