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Diagnostic and Therapeutic Mesenchymal Stem Cells for Breast Cancer Treatment

Introduction

It is estimated that there will be approximately 229,000 new breast cancer cases in the US in 2012. The recent trends towards improvement in breast cancer mortality rate are largely due to increased detection of early stage disease, while therapeutic options for advanced stage breast carcinomas remain limited. Breast cancer is a heterogeneous disease, and molecular signatures correlating with metastatic behavior and clinical outcome have been identified [1-4]. Sorlie and Perou [2] provided a distinctive “molecular portrait” of breast carcinomas based on variations in gene expression patterns, classifying tumors into five major subtypes (basal, ERBB2, luminal-A, luminal-B and normal-like). Clinical outcome was seen to be distinctly different between subtypes, with both recurrence free and overall survival worst in Basal-like and HER-2 positive tumours and the best prognosis being recorded in Luminal A tumours. 5-year survival rates are also intrinsically linked to the stage of disease at diagnosis, with patients with distant metastases at presentation having a significantly decreased survival rate. Although there has been a decrease in breast cancer death rates overall, it is anticipated that almost 40,000 people will die
from the disease in 2012, highlighting the need for novel therapies for metastatic disease[5]. The potential for use of Mesenchymal Stem Cells (MSCs) as cellular vehicles for tumor targeted delivery of therapeutic agents has generated a significant amount of interest in this context. MSCs have the proven ability to home to the site of multiple tumor types and their metastases following systemic delivery [6-8]. In the context of breast cancer, MSCs have been shown to migrate to primary tumors, lymph node and lung metastases following systemic administration in animal models [9-16]. The ability of MSCs to home to multiple metastases, and microscopic tumours [17] holds tremendous promise for the detection and treatment of advanced breast cancer.

**MSC Homing and Immune Regulation**

The microenvironment of a tumor is very similar to a wound site, which is thought to be the basis of MSCs ability to home to tumors. The tumor microenvironment is considered a site of chronic inflammation [18], supporting recruitment and engraftment of MSCs through secretion of soluble factors such as stromal-derived growth factor-1α (SDF-1α/CXCL12), monocyte chemoattractant protein-1 (MCP-1/CCL2), epidermal growth factor (EGF), vascular endothelial growth factor-A (VEGF-A), fibroblast
growth factor (FGF), platelet-derived growth factor (PDGF), IL-8, IL-6, granulocyte-macrophage colony-stimulating factor (GM-CSF), granulocyte colony-stimulating factor (G-CSF), Ang1, haematopoietic growth factor (HGF), transforming growth factor-β1 (TGF-β1) and urokinase-type plasminogen activator (uPA) [10, 19-23]. A recent study in a murine model of pancreatic cancer further highlighted the role that the degree of inflammation plays in the level of MSC recruitment, where treatment with an anti-inflammatory agent resulted in reduction of MSC engraftment in the tumor [24]. The process of MSC mobilization to the tumor is thought to be regulated similarly to leukocyte migration through integrins and adhesion molecules [25]. MSCs express a wide range of molecules including growth factors, chemokines, adhesion molecules and toll-like receptors (TLRs) on their surface [25-31]. They are known to functionally express a range of chemokine receptors and c-met, which has been increasingly linked to tumor tropism [26, 28-30]. Along with their tumor homing ability, MSCs seem to be able to bypass the immune system, so can essentially act as stealth vehicles, staying below the radar of the immune system and delivering agents directly to the tumor site. MSCs have been shown to interact with almost all cells of the innate and adaptive immune system [32]. In the adaptive immune system MSCs support survival of T cells in a quiescent state and also
suppress proliferation of activated T cells. They are also thought to suppress B cells, either directly, or indirectly through T cell suppression. In the case of the innate immune system, MSCs inhibit dendritic cell, NK cell and neutrophil function. They primarily influence dendritic cells, through inhibition of maturation, antigen presentation and secretion of pro-inflammatory compounds [32].

**Stem Cells that have combined therapeutic and Diagnostic Functions**

A range of approaches have been successfully employed to harness MSCs for cancer therapy in animal disease models, as discussed in Chapter 1. However, despite the extent of active research in this area, some fundamental questions remain to be answered. Although the tumor tropism of MSCs is generally accepted, the level of engraftment is no doubt a feature affected by each aspect of study design from variation in MSC isolation protocols, source, and characterization, to time course of delivery and time point of analysis or study endpoint, and features of the microenvironment created by the tumor in question. The ability to address these issues will be dependent upon effective detection and tracking of MSC behavior following introduction. The majority of research in this area initially employed analysis techniques for detection of MSCs ex vivo following animal necropsy and
tissue harvest. This included detection of fluorescently labeled MSCs by microscopy, the use of sex mismatched cells or reporter genes for detection by Immunohistochemistry or PCR. This was very useful initially to confirm the presence of MSCs in target tissues, however as research in this field progressed it became abundantly clear that MSC migration towards tumors is a dynamic process, with the cells often temporarily residing in other tissue en route, so the time point of necropsy could be critical. The ability to dynamically image MSCs as they migrate following systemic injection will be crucial to determine
1) the proportion of injected MSCs that reach the tumor target
2) timing of maximal tumor-specific engraftment
3) degree of ectopic engraftment in non-target tissues
4) persistence and differentiation fate of injected MSCs

A variety of approaches to modify MSCs for detection have been employed and fit within the broad categories of direct labelling, or indirect labelling through expression of reporter genes [33, 34].

Direct labelling employs incorporation of quantum dots or fluorophores into cells for optical fluorescence imaging, superparamagnetic iron oxide particles (SPIO) for magnetic resonance imaging (MRI), or radionuclides for single photon
emission computed tomography (SPECT) or positron emission tomography (PET). Reporter gene strategies have the advantage that detection indicates the presence of a viable cell and can provide more functional information, whereas direct cell labelling can result in false positive signals due to labelled cell uptake by phagocytic cells. Also, a reporter gene can be driven by a pathway specific promoter to give a signal only if a particular event takes place e.g. proliferation or differentiation.

Bioluminescence (BLI) is the most common approach for tracking stem cell fate in small animals, with Firefly Luciferase(fLuc) and Renilla Luciferase(rLuc) being the two most common reporter genes. BLI is highly sensitive, non-destructive and quantitative. However, this modality is currently restricted to small animal imaging due to the limited tissue depth that can be assessed, and there is increasingly a move towards more clinically relevant imaging modalities that can be efficiently translated to the patient setting. However each modality has inherent strengths and weaknesses. PET or SPECT for MSC tracking employs a reporter gene in conjunction with a radionuclide, or direct labelling with tracers such as $^{111}$Indium oxine. However, concerns have been raised that this type of labelling may have a detrimental effect on the cells, with one study reporting significant cell death
observed two weeks following labelling with $^{111}$In, although the MSCs showed no negative effects at earlier timepoints [35]. PET is highly sensitive and quantitative, and can be combined with CT to accurately localise radiotracer uptake. PET is more sensitive than MRI but of lower resolution. MRI offers spatial resolution but there is the potential toxicity of contrast agents used. The signal diminishes as the cells divide, and no information regarding cell viability is provided. Indeed the choice of any tracer or reporter gene requires confirmation that it does not significantly alter the biological properties and differentiation capacity of the stem cells.

Each of the approaches described has been applied to MSCs across a range of disease models [33, 34]. In murine models of breast cancer, systemically infused MSCs have been detected using BLI, SPECT and MRI (Table 1).

BLI has been the most commonly used approach in breast cancer models thus far [36-38], with the majority utilising cancer cells labelled with rLuc and MSCs labelled with fLuc. Administration of D-luciferin and coelenterazine then supports imaging the fLuc and rLuc labelled cells respectively. In all cases, breast tumor-targeted MSC engraftment was detected at varying timepoints. Using BLI, Kidd et al. [39] detected co-localisation of MSCs with
breast tumors from day 6 following infusion, but continuing to increase at day 12.

MRI and SPECT, which would be suitable for translation to the clinic have also been employed in this setting [11, 40]. MSCs were induced to take up iron nanoparticles prior to IV infusion in a model of metastatic breast cancer and images acquired 1hr and 24hrs later showed co-localization of the cells with lung metastases [40]. As few as 1,000 MSCs carrying iron oxide nanoparticles could also be visualised one month after co-injection with breast cancer cells. This study reported no detrimental effect of the nanoparticles on MSC differentiation potential, proliferation, survival or migration [40]. The absence of a false positive signal arising from dead or phagocytosed cells was also demonstrated.

One of the earliest studies reporting tracking of MSCs in vivo in conjunction with a therapeutic response was by Khakoo et al [41], although in this case the MSCs were not engineered for therapy, but demonstrated intrinsic anti-neoplastic properties. The SPIO-labelled cells were dynamically imaged homing to Kaposi sarcoma using MRI [41]. While the number of studies using live imaging of MSCs is certainly increasing there remain relatively few that describe delivery of a therapeutic payload to tumors in conjunction with dynamic imaging of the delivery vehicle (Table
The majority of these have been performed on models of glioma, with BLI often the imaging modality of choice. Along with tracking engraftment, the use of selective promoters has been employed to generate functional information about cell fate following engraftment. For example, a recent report described the use of MSCs expressing a reporter or therapeutic gene under the selective control of the Tie2 promoter [51]. In this setting, MSCs expressed HSV-tk or red fluorescent protein only when they developed endothelial-like characteristics. This approach showed efficacy in both pancreatic and breast tumour models in vivo [51]. Harnessing the differentiation capacity of MSCs for tissue regeneration has also been employed in the prostate cancer setting, where murine MSCs were engineered to deliver urokinase-type plasminogen antagonist amino terminal fragment (hATF) to impair osteolytic prostate cancer cell progression in bone and repair bone lesions (Table 2) [43]. Progression of the luciferase expressing PC-3 tumours was tracked using BLI, with significant inhibition of tumour progression observed when the cells were co-injected with MSC-hATF. The osteogenic potential of the MSCs was unaffected and new areas of bone formation were detected in 60% of animals [43]. Along with alternate luciferase-based imaging of the MSCs and prostate cancer cells, this study combined the properties of MSCs as
vehicles for the angiostatic agent with their osteogenic potential.

MSCs engineered to express TRAIL were successfully tracked migrating to highly invasive gliomas in vivo, using fLuc bioluminescence. Interestingly, in order to follow stability and duration of S-TRAIL secretion in vivo, a fusion protein was also created with GLuc. This supported tracking of MSC migration and also of the therapeutic agent simultaneously, with a potent anti-tumor effect observed [48]. A similar approach has also been employed in the context of neural stem cells, using a range of viral fusion variants containing therapeutic TRAIL and diagnostic luciferase [52]. This supported dynamic imaging of the cells and effective therapy for highly malignant gliomas.

The use of fLuc and BLI has also been employed in prostate cancer and glioma models to track migration of MSCs expressing HSV-tk [49, 50]. Following successful serial imaging, administration of the prodrug ganciclovir (GCV) resulted in a significant therapeutic effect, which was even further enhanced when MSCs were engineered to produce the retroviral vector locally at the tumour site [49]. Expression of HSV-tk has the dual capacity for imaging by PET in combination with $[^{18}\text{F}]$FHBG, and therapy in the presence of GCV. This was exploited for MSC-based targeted imaging and treatment of glioma [46]. Anti-angiogenic agents
coupled with bioluminescent or live fluorescent imaging have also proven successful [43, 44]. Neural stem cells have also been employed in this setting for the treatment of glioma, where the cells expressed the anti-angiogenic protein thrombospondin along with bioluminescent proteins for dynamic imaging [53].

The first report of MSC-mediated therapy of cancer was by Studeny et al. [54], where the cells were engineered to secrete Interferon-β (IFN-β) at therapeutic levels locally at the tumor site resulting in significantly prolonged animal survival. More recently this was expanded to BLI tracking of MSCs in real time in combination with IFN-β secretion [24]. The same group have also used a similar approach where the tumour cells were engineered to express luciferase to support tracking of tumour burden, although the MSCs were not visualized in this case [55]. Other studies have also shown effective imaging of virus production by MSCs [56] or tumour burden [57] in response to therapeutic MSCs using BLI.

We recently employed SPECT imaging in combination with radionuclide therapy in this setting. In this case MSCs were not directly labelled with a radionuclide, but were engineered to express the Sodium Iodide Symporter (NIS) gene, which allows cells to concentrate the tracer, and so as a reporter gene indicates the presence of a viable cell [11]. This approach had previously been described for MSC tracking following systemic
infusion in vivo, although no therapeutic element had been included [58]. Following systemic administration of the radiotracer technetium-99m pertechnetate ($^{99m}$TcO$_4$), we found MSCs localised to breast tumors from Day 3 after IV infusion, although significant ectopic engraftment was also detected at this time point (Figure 1).

In control animals that had received no injection of MSC-NIS, a pattern of uptake correlating with native NIS expression was observed, with $^{99m}$TcO$_4^-$ concentrated in the thyroid/salivary gland and stomach (Figure 1A). The bladder was also visible in some images as a result of tracer excretion in urine. Three days following direct intratumoral injection of MSC-NIS robust uptake of tracer at the tumor site was observed (Figure 1B). Following IV administration of MSC-NIS, animal intestines, chest cavity and a weak image of the right flank tumor were visible (Figure 1C). By Day 14, uptake of tracer was visible at the site of the tumor with a significant reduction in accumulation in non-target tissue observed (Figure 1D). Based on ROI readings, levels of tracer accumulation in areas of interest were expressed as a percentage of total administered dose. This revealed an almost 9-fold increase in signal accumulation at the tumor site at Day 14 following MSC-NIS infusion compared to Day 3. RQ-PCR analysis of tissues harvested following γ-camera imaging supported this data,
with robust human NIS expression accumulating and persisting at
the tumor site while depleting in non-target organs.
The serial imaging 3, 7, 10 and 14 days following intravenous
MSC-NIS injection in animals revealed optimal radiotracer signal
at the tumor site at day 14. Following establishment of the
optimal balance between ectopic and tumor-targeted engraftment,
an ablative dose of $^{131}$I was administered (Figure 2).

In control animals (no MSC injection) and those that received
MSC-NIS IV without $^{131}$I therapy, tumor volume continued to
increase rapidly throughout the 8 weeks of monitoring, with no
significant difference in volume detected between the two groups.
In animals that received IT injections of MSC-NIS followed by
saline or $^{131}$I, there was an apparent reduction in the rate of
tumor growth from week 3 onwards (5 weeks following MSC
injection), although no significant difference in tumor volume
between treated and untreated animals was detected. However,
histological analysis of tumour tissue harvested following the
monitoring period revealed extensive necrotic areas in tumors
harvested from animals that received $^{131}$I therapy irrespective of
the route of MSC-NIS delivery. The greatest impact of
intervention was observed in animals that received an IV
injection of MSC-NIS followed by $^{131}$I 14 days later, which
resulted in a significant reduction in tumor growth rate and
A cytostatic effect was observed, with resulting tumor volumes approximately 75% smaller than their counterpart controls (Figure 2) [11]. This study further highlighted the importance of tracking MSC migration in real time, and the advantage of being able to perform a pretherapy scan to confirm tumor targeted MSC-NIS engraftment before administration of $^{131}$I. Knoop et al. [45] also described successful exploitation of the dual role of NIS as both a reporter and therapeutic gene following systemic infusion in Hepatocellular carcinoma bearing animals. In this case both γ-camera imaging and PET were employed to track the MSCs using $^{123}$I and $^{124}$I respectively. Imaging was performed at only one timepoint following three cycles of MSC-NIS injection at four day intervals, although the retention time of the tracer within the tumour was determined by serial scanning on the same day. For tumour therapy, in this case three cycles of MSC-NIS were administered at intervals and $^{131}$I administered 48hrs following the last injection. The entire treatment cycle was then repeated, followed again by one further injection of MSC-NIS and $^{131}$I therapy. This resulted in a significant delay in tumour growth, with a demonstrated decrease in proliferation and red blood vessel density [45]. Although a very positive outcome, it would be interesting to determine whether the number of MSC injections could be reduced if the timing of therapy was
optimised based on imaging. However, the additional cycles of $^{131}$I may in fact be beneficial. Irradiation has been shown to increase tumor engraftment of MSCs in breast and other cancers through apoptosis and increased release of inflammatory agents [37, 59]. Repeated injection with $^{131}$I may have improved engraftment of subsequent doses of engineered MSCs. Indeed, radiotherapy is frequently a component of breast cancer therapy and so could work in combination with MSC based gene delivery to support improved targeting of MSCs to tumors.

It is advantageous to be able to use the same protein product both as a reporter and therapeutic gene. This is also the case with HSV-tk, although there are issues with immunogenicity. In contrast, NIS is a normal human protein and so is non-toxic and non-immunogenic. The extensive safety record of radioiodide therapy in the treatment of thyroid disease is a major advantage to the use of NIS as a therapeutic gene [60]. In the context of breast cancer, significant inhibition of thyroidal uptake of $^{131}$I can be achieved through T$_3$ supplementation prior to and during $^{131}$I therapy [60, 61]. A range of isotopes are efficiently transported by NIS, and are readily available and approved for clinical purposes. Also, gamma camera technology is more widely accessible than more expensive PET scanners.

An important point to highlight here is that in the majority of cases, optimal MSC engraftment was not detected until a number of
days following IV delivery of the cells. In the example shown in Figure 1, and many other studies besides, localisation in the lungs and elsewhere was detected at early stages following MSC delivery, with ectopic engraftment decreasing and tumor-targeted engraftment increasing over time. Kidd et al [39] reported that MSCs co-localised at sites of tumour development independent of immunocompetence from Day 6, with levels of engraftment further increased by D12. Uchibori et al. [49] also demonstrated transient detection of MSCs in high perfusion organs just after injection, with the tumor-targeted MSC signal found to peak at Day 10 following injection. The use of a strategy that can support both imaging and therapy is highly advantageous in this setting, as it provides the ability to perform serial imaging and detect optimal tumor-targeted engraftment before proceeding to activation of the anti-neoplastic properties of the cells. However, very little is known regarding the long term fate, differentiation status or role of MSCs following engraftment in an established tumour. Extended time course studies are needed in this area and dynamic imaging will be key to further understanding the biology of the cells in this setting. The use of MSCs expressing suicide genes for therapy will have the added safety advantage that the cells will not persist following therapy.
MSCs hold great potential as therapeutic vehicles for the treatment of metastatic breast cancer as a result of their remarkable tumour-homing capacity and immune privilege. MSCs target a range of cancer types so this has potential applications in many diseases, and considering almost half a million deaths from cancer are projected to occur in the United States alone in 2012, this holds great promise. There are concerns regarding a potential protumorigenic role of MSCs and this has been a subject of much debate [19, 32, 36, 62, 63]. The main foci for concern are based on 1) MSC transformation potential and de novo tumour formation 2) role of MSCs as supportive stroma (carcinoma associated fibroblasts) within the primary tumour microenvironment and 3) immunosuppressive qualities of MSCs supporting tumour cell evasion of the host immune system. The majority of studies to date have shown that human MSCs are stable [64, 65], while their murine/rat counterparts appear to be more prone to transformation [65-68]. One study that did report spontaneous transformation of human MSCs [69] was later retracted after it was suspected that the phenomenon was due to cross-contamination artefacts [70]. There are currently over 250 clinical trials utilising MSCs ongoing worldwide (clinicaltrials.gov; clinicaltrialsregister.eu; [7]). Most trials published to date have evaluated the safety and
efficacy of MSCs within the context of non-cancerous conditions with no acute or long term adverse effects reported so far, including no reports of carcinogenesis. There is no doubt however, that it will be critically important to stringently monitor MSC genotype and phenotype prior to clinical application. Co-injection of MSCs and breast cancer cells has been shown to increase tumour growth and metastasis [71], and using a similar approach, MSCs were demonstrated to promote hormone independence which could have important clinical implications[72]. They have been shown to differentiate into CAFs under the influence of local stimuli [73, 74] and have been implicated in promotion of tumor cell entry into bone marrow and development of bone metastases [75, 76]. Despite this, the majority of studies using exogenously introduced MSCs have shown no protumorigenic effect of control or engineered MSCs following infusion into animals with pre-established tumors [9, 11, 24, 41, 48, 77-82]. Understanding interactions between breast cancer cells and MSCs in the primary tumor microenvironment will be fundamental to determining their true potential in the clinical setting. It is of interest that two factors implicated in MSC migration, CCL2 and CXCL12 [10, 15, 37, 83, 84], are secreted at highest levels by tumor stromal, rather than epithelial cells [10]. This suggests that mixed stromal-epithelial xenografts in murine models, which would more closely recapitulate the disease in
patients [85], may actually result in further improvement of the level of tumor-targeted MSC engraftment. Attempts are also being made to improve tumor tropism and infiltration through modification of the MSC surface [86].

There are a number of fundamental questions that remain to be answered, and the ability to track MSC migration, engraftment and biological behaviour through in vivo imaging in real time will be central to progression of this exciting approach to cancer therapy.
<table>
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<tr>
<th>Cell Labels</th>
<th>Substrate</th>
<th>Imaging Modality</th>
<th>Time following MSC Injection</th>
<th>Observations</th>
<th>Reference</th>
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<tr>
<td>4T1 – rLuc</td>
<td>Coelenterazine</td>
<td>BLI</td>
<td>Day 1, 4, 6</td>
<td>MSC engraftment enhanced in irradiated tumours with optimal effect at Day1</td>
<td>[37]</td>
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<tr>
<td>MSC- fLuc</td>
<td>D-luciferin</td>
<td></td>
<td></td>
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<td>4T1 syngeneic-rLuc</td>
<td>Coelenterazine</td>
<td>BLI</td>
<td>Days 0.5, 6 and 12 Days 1, 3, 11, 29</td>
<td>MSCs co-localised at sites of tumour development independent of immunocompetence from Day6, further increased by D12</td>
<td>[39]</td>
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<td>MDA-MB-231 xenogeneic unlabelled</td>
<td>Coelenterazine</td>
<td>BLI</td>
<td>Days 1, 4, 6, 9, 11 SC Tumour: Days 1, 4, 6, 8, 12, 14</td>
<td>MSC localized, survived and proliferated in tumours; peak engraftment seen at D11-D14.</td>
<td>[38]</td>
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<tr>
<td>MSC- fLuc</td>
<td>D-luciferin</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>4T1 – rLuc-mRFP</td>
<td>Coelenterazine</td>
<td>BLI</td>
<td>Lung mets: 1hr, Days 1, 4, 6, 9, 11 SC Tumour: Days 1, 4, 6, 8, 12, 14</td>
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<td>D-luciferin</td>
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<tr>
<td>4T1 – rLuc-mRFP</td>
<td>Tc-99m tracer</td>
<td>SPECT</td>
<td>Day 3, 7, 10, 14</td>
<td>Detectable at tumour by D3, optimal targeted engraftment at D 14</td>
<td>[11]</td>
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<tr>
<td>MSC- NIS transgene</td>
<td></td>
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Table 1: Reports of dynamic imaging of MSCs in real time during breast tumour-targeted migration; eGFP: Enhanced Green Fluorescent Protein; BLI: Bioluminescent Imaging; MRI: Magnetic Resonance Imaging; SPECT: Single Positron Emission Computed Tomography; rLuc: renilla luciferase; mRFP: monomeric Red Fluorescent Protein; fLuc: firefly luciferase
<table>
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<tr>
<th>Tumor Model</th>
<th>MSC Reporter</th>
<th>Substrate</th>
<th>Imaging Modality</th>
<th>Therapeutic Agent</th>
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<td>Glioma</td>
<td>HSV-tk</td>
<td>[18F]FHBG</td>
<td>PET</td>
<td>HSV-tk + GCV</td>
<td>[46]</td>
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<td>Prostate</td>
<td>Luciferase</td>
<td>D-luciferin</td>
<td>BLI</td>
<td>Urokinase-type plasminogen antagonists</td>
<td>[43]</td>
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<td>Colon</td>
<td>Luciferase</td>
<td>D-luciferin</td>
<td>BLI</td>
<td>Bi-specific α-CEA diabody</td>
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<td>BLI</td>
<td>TRAIL</td>
<td>[48]</td>
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<td>Prostate</td>
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<td>BLI</td>
<td>HSV-tk + GCV</td>
<td>[50]</td>
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<tr>
<td>Glioma</td>
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<td>D-luciferin</td>
<td>BLI</td>
<td>HSV-tk + GCV</td>
<td>[49]</td>
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<td>Pancreatic</td>
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<td>BLI</td>
<td>IFN-β</td>
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<td>D-luciferin</td>
<td>Live fluorescent</td>
<td>Hemopexin-like protein (PEX)</td>
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<td>NIS</td>
<td>$^{123}$I $^{124}$I</td>
<td>γ-camera PET</td>
<td>NIS + $^{131}$I</td>
<td>[45]</td>
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<td>$^{99mTc}$O$_4$</td>
<td>SPECT</td>
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<td>Gastric</td>
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<td>N/A</td>
<td>Live Fluorescent MRI</td>
<td>External alternating magnetic fields</td>
<td>[47]</td>
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*Table 2: Reports of dual dynamic imaging of MSCs in real time with concurrent delivery of therapy; HSV-tk: Herpes Simplex Virus thymidine kinase; PET: positron emission tomography; BLI: Bioluminescent Imaging; fLuc: firefly luciferase; pLuc: Photinus pyralis Luciferase; MRI: Magnetic Resonance Imaging; SPECT: Single Positron Emission Computed Tomography; rLuc: renilla luciferase; mRFP: monomeric Red Fluorescent Protein;*
Figure 1: BazookaSPECT γ-camera imaging of mice after administration of 2 mCi/74 MBq technetium-99m pertechnetate (99mTcO4⁻). (A): Single coronal slice of volume rendering from a reconstructed SPECT image of a control animal-(MSC free) showing 99mTcO₄⁻ uptake in the thyroid and stomach, which express endogenous sodium iodide symporter (NIS). (B): Single transverse slice of volume rendering from a reconstructed SPECT image of a tumor 3 days after intratumoral injection of MSC-NIS, showing robust uptake of tracer. (C, D): Planar images of tumor bearing animals 3 days (C) or 14 days (D) after IV injection of MSC-NIS. (C): Revealed a pattern of uptake apparently correlating with the location of the intestines, and also diffuse uptake in the chest area. A weak image of the right flank tumor can also be observed. (D): Imaging 14 days after MSC-NIS injection revealed a clear image of the right flank tumor, with a significant reduction in ectopic concentration of tracer in nontarget tissue observed. Reprinted with permission from Dwyer et al. (2011).
**Figure 2:** In vivo radioiodine therapy of MDA-MB-231 breast tumor xenografts 14 days after injection of mesenchymal stem cell (MSC)-sodium iodide symporter (NIS). Animals received an intratumoral or intravenous injection of NIS-expressing MSCs followed by an i.p. dose of either $^{131}$I or saline 14 days later. Abbreviations: MSC, mesenchymal stem cell; NIS, sodium iodide symporter. Reprinted with permission from Dwyer et al. (2011).
References


