Mesenchymal stem cell (MSC)-mediated, tumor stroma-targeted radioiodine therapy of metastatic colon cancer using the sodium iodide symporter as theranostic gene

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Running title: MSC-mediated NIS gene delivery

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ABSTRACT

The tumor-homing property of mesenchymal stem cells (MSCs) allows targeted delivery of therapeutic genes into the tumor microenvironment. The application of sodium iodide symporter (NIS) as a theranostic gene allows non-invasive imaging of MSC biodistribution and transgene expression before therapeutic radioiodine application. We have previously shown that linking therapeutic transgene expression to induction of the chemokine CCL5/RANTES allows a more focused expression within primary tumors, as the adoptively transferred MSC develop carcinoma-associated fibroblast (CAF)-like characteristics. While RANTES/CCL5-NIS-targeting has shown efficacy in the treatment of primary tumors, it was not clear if it would also be effective in controlling the growth of metastatic disease.

Methods:
To expand the potential range of tumor targets we investigated the biodistribution and tumor recruitment of MSCs transfected with NIS under control of the RANTES/CCL5 promoter (RANTES-NIS-MSC) in a colon cancer liver metastasis mouse model established by intrasplenic injection of the human colon cancer cell line LS174t. RANTES-NIS-MSCs were injected intravenously followed by $^{123}$I-scintigraphy and $^{124}$I-PET imaging as well as $^{131}$I therapy.

Results:
Results show robust MSC recruitment with RANTES/CCL5-promoter activation within the stroma of liver metastases as evidenced by tumor-selective iodide accumulation, immunohistochemistry and real-time PCR. Therapeutic application of $^{131}$I in RANTES-NIS-MSC-treated mice resulted in a significant delay in tumor growth and improved overall survival.
Conclusion:

This novel gene therapy approach opens the prospect of NIS-mediated radionuclide therapy of metastatic cancer after MSC-mediated gene delivery.

*Key words:* Sodium iodide symporter, mesenchymal stem cells, hepatic metastases, colon cancer, gene therapy, RANTES
INTRODUCTION

Effective control of metastatic disease represents a central challenge in the treatment of solid tumors. Colorectal cancer is the third most common cancer world-wide with the liver being the most common metastatic site. While the prognosis of resectable colorectal liver metastases was improved in the recent years, 5-year survival rates after resection are still not higher than 25% to 40%, and survival rates have remained poor for unresectable disease (1).

An emerging tumor therapy approach is based on the use of adoptively transferred mesenchymal stem cells (MSCs) as vehicles engineered to express specific reporter and/or therapy genes (eMSC). This approach utilizes the innate ability of MSCs to migrate to damaged tissue in the course of tissue repair (2). Tumors are seen by the body as chronic, never healing wounds that drive continuous tissue remodeling thus providing the basis for the marked tumor tropism of MSCs (3). We and others have harnessed this biology by using eMSC to deliver anti-cancer gene products into tumor stromal environments (4-10). Adoptively transferred MSCs are actively recruited to growing tumors where they contribute to the formation of the "benign" stromal compartment containing a variety of cell types, including endothelial cells, smooth muscle cells, and pericytes/carcinoma-associated fibroblasts (CAFs) that provide important support for the growth of solid tumors. However, MSCs also contribute to normal tissue homeostasis and therefore may also emigrate to non-tumor environments under these conditions. In order to reduce potential side effects of transgene expression in non-tumor-associated tissues, the therapeutic utility of eMSCs has been enhanced by the use of tumor stroma-specific gene promoters linked to unique differentiation pathways activated as MSC respond to tumor microenvironments. For example, the Tie2
promoter/enhancer is activated as MSC respond to angiogenic signals within tumor environments (7). MSC engineered to express the suicide gene herpes simplex virus thymidine kinase (HSV-TK) under control of the Tie2 promoter/enhancer showed selective expression of transgenes following systemic injection of the eMSC into mice with growing breast, pancreatic or liver tumors (7, 9). While this approach was effective in targeting large primary tumors, the Tie2-based strategy showed low efficacy in the treatment of tumor metastases. An alternative targeting approach was therefore developed using the RANTES/CCL5 promoter to drive transgene expression in MSCs. In the course of differentiation into CAFs, MSCs induce expression of the chemokine RANTES (11, 12). eMSC engineered to express HSV-TK under control of the RANTES promoter in concert with GCV treatment led not only to a significant reduction in the growth of primary pancreatic carcinoma, but also dramatically reduced incidence of metastases in a pancreatic cancer model (6). Importantly, it was not possible to distinguish if this therapy effect was due to an overall reduction in metastases, or direct targeting of individual metastases.

More recently, to enhance the therapeutic potential of MSC-mediated gene therapy, a more advanced therapy gene was applied that makes use of the selective delivery of radionuclides for imaging and therapy. The sodium iodide symporter (NIS) protein is responsible for the active uptake of iodide from the blood into the thyroid gland, and as such, forms the molecular basis for the diagnostic and therapeutic use of radioiodine in the management of differentiated thyroid cancer (13). One of the major advantages of NIS as a therapy gene is its dual function as therapy and reporter gene (13-16). MSCs engineered to express NIS can be monitored by whole body imaging using $^{123}$I-scintigraphy/SPECT or $^{124}$I-PET allowing direct, non-invasive in vivo imaging of MSC
biodistribution and functional transgene expression \((16, 17)\). Importantly, a robust therapeutic effect can be delivered through \(^{131}\text{I}\) or \(^{188}\text{Re}\) application \((4, 5)\). In the present study, the efficacy of RANTES-based eMSC targeting of NIS expression was evaluated in an experimental mouse model of colon cancer liver metastases. The model was designed to exclude potential effects on the primary tumor in order to provide information about the efficacy of this therapeutic approach on smaller, metastatic tumor targets.

**MATERIALS AND METHODS**

**Cell Culture**

The establishment and characterization of MSCs has been described previously \((4, 6, 18)\). These cells have been previously shown to maintain their capacity to differentiate, and show migration that parallels that seen with primary cells \((4, 6, 18)\). The ease of expansion of clonal populations allows well controlled experimental studies. Plasmids and their synthesis as well as the establishment of stably transfected cell lines have been described previously \((4, 5)\).

The human colon carcinoma cell line LS174t (ATCC CCL188) was cultured in RPMI (Invitrogen/Life technologies, Darmstadt, Germany) supplemented with 10% fetal bovine serum \((v/v; \text{PAA})\) and 1% penicillin/streptomycin. The cell lines were maintained at 37°C and 5% CO\(_2\) in an incubator with 95% humidity.

**Establishment of a Hepatic Colon Cancer Metastases Mouse Model**

The experimental protocol was approved by the regional governmental commission for
animals (Regierung von Oberbayern). Fully anesthetized female CD-1 nu/nu mice (Charles River, Sulzfeld, Germany) were placed in the right lateral position, after skin desinfection a 0.5 cm cut was made at the left subcostal region. 50 µl of tumor cell suspension (1x10⁶ cells in 1xPBS) were then injected at the upper splenic pole using a 27-gauge needle. Abdominal wall and skin were then sutured separately using Monosyn® 5/0 fiber. 48 h later the abdominal wall was re-opened at the same site and a splenectomy was performed, followed by suturing of abdominal wall and skin. Mice were pre- and post-treated with Carprofen (5 mg/kg) to minimize wound pain. The induction of liver metastases with this technique was highly reproducible; histological liver studies showed that 90% of injected mice developed disseminated liver metastases. Animals were maintained under specific pathogen-free conditions with access to mouse chow and water ad libitum.

**MSC Application and Radionuclide Biodistribution Studies in vivo**

Application of eMSC was initiated five days after intrasplenic tumor cell injection, before metastatic disease was macroscopically visible. In addition, mice were pretreated with thyroid hormone L-T4 (levothyroxine) as described previously (4). Wild-type (WT)-MSCs or RANTES-NIS-MSCs were applied via the tail vein (5 x 10⁵ cells/500µl PBS). Two groups of mice were treated as follows: (a) three intravenous (i.v.) applications of RANTES-NIS-MSC in three day intervals (n=22); (b) three i.v. applications of WT-MSC in three day intervals (n=7). As an additional control, in a subset of mice injected with RANTES-NIS-MSC (n=7) the specific NIS-inhibitor sodium-perchlorate (NaClO₄, 2 mg/mouse) was injected intraperitoneally (i.p.) 30 min prior to radionuclide administration. 48 h after the last MSC application, 18.5 MBq ¹²³I was injected i.p. and
radionuclide biodistribution was assessed using a gamma camera equipped with UXHR collimator (Ecam, Siemens, Germany) as described previously (19, 20). Small-animal PET imaging was performed in a subgroup of mice treated with RANTES-NIS-MSC (n=8) receiving a dose of 12 MBq $^{124}$I 48 h after the last MSC application, as described previously (4).

**Ex Vivo Analyses of Tumor and Non-Target Tissues**

For ex vivo biodistribution studies, mice were injected with RANTES-NIS-MSCs (n=15) or WT-MSCs (n=5) as described above, followed by i.p. injection of 18.5 MBq $^{123}$I. A subset of RANTES-NIS-MSC injected mice (n=5) were treated with NaClO$_4$ prior to radionuclide administration as an additional control. Three hours after radioiodine injection, levels of $^{123}$I in liver and non-target organs were analyzed ex vivo as described previously (4, 5).

At the end of the imaging procedure, immunohistochemical and immunofluorescence staining procedures for the expression of NIS and SV40 large T Ag as well as quantification of cellular proliferation (Ki67) and blood vessel density (CD31) were performed as described previously (5).

Analysis of NIS mRNA expression using quantitative real-time PCR were performed at the end of the imaging procedure as outlined above. Total RNA was isolated from the liver and other tissues using the RNeasy Mini Kit (Qiagen, Hilden, Germany) according to the manufacturer’s recommendations and quantitative real-time PCR (qPCR) was performed as described previously (21).

**Radioiodine ($^{131}$I) Therapy Studies in vivo**
Following a 10-day L-T4 pretreatment as described previously (4, 5), two groups of mice were established that each received 55.5 MBq $^{131}$I (sodium iodide; GE Healthcare Buchler GmbH, Braunschweig, Germany) 48 h after three RANTES-NIS-MSC (RANTES-NIS-MSC + $^{131}$I, n=15) or WT-MSC (WT-MSC + $^{131}$I, n=15) applications given in two-day-intervals (each $5 \times 10^5$ cells/500 µl PBS). 24 h later we applied two more rounds of two MSC ($5 \times 10^5$ cells) injections (in two-day-intervals) followed by 55.5 MBq $^{131}$I 48 h later (Fig. 5A). As control, one further group of mice was treated with saline instead of $^{131}$I after injection of RANTES-NIS-MSC (n=15). By protocol, mice were sacrificed when healthy liver tissue reached less than 20%, or in case of weight loss of more than 10% of initial weight, or when impairment of breathing, drinking or eating behavior was observed.

**Magnetic Resonance Imaging (MRI)**

For MRI examinations, animals were anaesthesized by i.p. injections of ketamine (Inresa Arzneimittel, Freiburg, Germany) 100mg/kg body weight) and xylazine (Bayer, Leverkusen, Germany) 10mg/kg body weight) and a 27-gauge tail vein catheter was placed for subsequent contrast media administration. MRI was performed on a clinical 3 Tesla system (Magnetom Skyra, Siemens Healthcare, Erlangen, Germany) with animals in prone position, using a clinical wrist coil (Siemens Healthcare, Erlangen, Germany). MRI acquisition was performed using a brief imaging protocol with T1 sequences in axial and coronal view for optimal delineation of intrahepatic tumor manifestations. T1-weighted FLASH3D sequences were acquired pre-contrast and after a standardized i.v. manual bolus injection of 100 µl of Gadolinium-based contrast medium (Primovist®, Bayer Healthcare AG, Berlin, Germany). Sequence details were TR=5.74 ms; TE=2.26
ms; ST=1 mm; α=10°; matrix size 416×416; reconstructed matrix 832×832; field of view 150×150 mm²; spatial resolution 0.18×0.18×1 mm³; 80 slices; acquisition time 142 sec.

**Statistical Methods**

Statistical significance of in vitro experiments was tested using Student’s t test. Statistical significance of the in vivo experiments has been calculated using the Mann-Whitney U test.

**RESULTS**

**Radioiodine in vivo imaging studies**

To evaluate the ability of eMSC to target early stages of colon cancer liver metastases, eMSC injections via the tail vein were started five days after intrasplenic injection of the human colon cancer cells LS174t before visible metastases developed, biodistribution of RANTES-NIS-MSC was then determined by ¹²³I-scintigraphy. Enhanced iodide uptake activity was detected in the liver area two hours after injection of 18.5 MBq ¹²³I (Fig. 1A), which resulted in a maximum uptake of 12.1 ± 2.6 % ID/g with a biological half-life of 2.9 h, and a tumor absorbed dose of 63.2 mGy/mBq (Suppl. Fig. 1). In contrast, after injection of WT-MSC no liver-specific iodide accumulation was detected (Fig. 1C). To further demonstrate NIS-specificity of iodide uptake in the liver region, a subset of RANTES-NIS-MSC-treated mice received NaClO₄ 30 min prior to radioiodine injection, which resulted in a complete blockade of iodide accumulation in liver metastases, in addition to thyroid gland and stomach (Fig. 1B), which represent physiologically NIS-expressing organs. The high iodide accumulation seen in the bladder is a result of renal iodide excretion. To improve the resolution of radioiodine uptake in the liver area, and to
more clearly differentiate between hepatic and gastric iodide accumulation, additional PET imaging studies were performed using $^{124}$I. Three-dimensional data were subsequently generated using iterative reconstructions of list mode data (0-40min), which provided better anatomical definition. 48 hours after the last RANTES-NIS-MSC administration, 12 MBq $^{124}$I were applied and selective iodide accumulation was detected in single metastases three hours after $^{124}$I application (Fig. 1D, E). In 70% of RANTES-NIS-MSC-treated mice a maximum uptake of 16.2 ± 3.5 % ID/g was measured in single nodules.

**Ex vivo radioiodine biodistribution study**

Ex vivo gamma counter analysis of radioiodine biodistribution was performed which confirmed increased iodide uptake (approximately 6.1 ± 1.1 % ID/g) in the liver of RANTES-NIS-MSC-treated mice 3 hours after $^{123}$I injection (Fig. 2). In contrast, mice injected with WT-MSCs showed no significant hepatic iodide uptake and no significant iodide uptake levels were observed in non-target organs (Fig. 2). In both groups, the thyroid gland and the stomach accumulated approx. 40% and 39% $^{123}$I ID/organ, respectively, resulting from endogenous expression of NIS in these organs (data not shown). In additional control mice injected with RANTES-NIS-MSC, administration of the competitive inhibitor perchlorate resulted in a blockade of iodide uptake in liver (Fig. 2) as well as thyroid gland and stomach (data not shown).

**Immunohistochemical analysis of eMSC / transgene biodistribution**

SV40 large T Ag was used to immortalize the MSCs and could thus be used as target to assess the biodistribution of eMSCs, while human NIS-specific antibodies allowed the
determination of RANTES promoter-induced NIS transgene expression. Paraffin-embedded tissues from liver and additional “non-target” organs (lung, kidneys) were processed for immunohistochemical staining using both sets of antibodies. In liver metastases of mice treated with RANTES-NIS-MSCs, NIS-specific immunoreactivity was confined to metastatic nodules, and no NIS-specific staining was seen in the surrounding normal liver tissue (Fig. 3A). This biodistribution pattern was confirmed by SV40 large T Ag-specific staining (Fig. 3C). Systemic injection of WT-MSCs showed no NIS-specific immunoreactivity in metastases or in normal liver tissue (Fig. 3B); However, SV40 large T Ag staining still demonstrated active WT-MSC recruitment into the metastatic nodules (Fig. 3D), thus supporting tumor-selective eMSC recruitment. Immunofluorescence analysis confirmed co-localization of NIS- and SV40 large T Ag staining in the stroma of liver metastases of tumor-bearing mice after injection of RANTES-NIS-MSC (Suppl Fig. 2A – C).

In non-target organs such as lung or kidney, no NIS-specific or SV40 large T Ag-specific staining was detected in mice treated with RANTES-NIS-MSCs (Suppl. Fig. 2D – G).

**Analysis of NIS mRNA expression**

Metastatic livers from mice treated with RANTES-NIS-MSCs revealed a 7.6-fold increased level of NIS mRNA expression as compared to metastatic livers from mice treated with WT-MSCs. As expected, additional treatment with the competitive NIS inhibitor perchlorate (NaClO₄) had no influence on NIS mRNA expression in metastases-bearing mice injected with RANTES-NIS-MSC (7.4-fold increase). In contrast, non-target organs like lung or kidney showed no NIS mRNA expression in RANTES-NIS-MSC- or WT-MSC-treated mice (Suppl. Fig. 3).
**131I therapy study**

The therapeutic effect of 131I was assessed using the therapy regimen optimized in previous studies (4, 5). The protocol is based on three cycles of eMSC injections followed by 131I (Fig. 4A). After systemic injection of RANTES-NIS-MSC followed by 131I injection a significantly improved survival of up to 14 days was observed as compared to control groups (Fig. 4A).

Growth of metastases was monitored by MRI starting before the first 131I application after three injections of eMSCs (10 days after intrasplenic tumor cell injection) when the tumor load in the liver was moderate (approximately 40%) (Fig. 4B). Over time, an exponential tumor growth was seen in the control groups (RANTES-NIS-MSC + NaCl or WT-MSC + 131I) as compared to the therapy group (RANTES-NIS-MSC + 131I) that showed a significantly reduced tumor growth. MRI images at day 19 showed a reduced hepatic tumor load of approximately 60% in the therapy group (RANTES-NIS-MSC + 131I) (Fig. 4C) as compared to a tumor load of at least 90% in the control group (RANTES-NIS-MSC + NaCl) (Fig. 4D). Mice in the control groups did not survive long enough to receive the final 131I or saline application.

At the end of experiments, animals were sacrificed, and their organs removed and characterized by immunofluorescence analysis for cellular proliferation (Ki67, green) and blood vessel density (CD31, red) markers. The results showed striking differences between therapy and control groups (Suppl. Fig. 4A – C). Mice treated with RANTES-NIS-MSCs followed by 131I showed decreased proliferation (Ki67: 23.3% ± 2.5%) and blood vessel density (CD31: 3.1% ± 0.2%), whereas control mice treated with RANTES-NIS-MSC followed by NaCl or treated with WT-MSCs followed by 131I revealed high
blood vessel density (RANTES-NIS-MSC + NaCl: CD31: 6.6% ± 0.7%; WT-MSC + 131I: CD31: 7.8% ± 0.8%) and high levels of proliferation (RANTES-NIS-MSC + NaCl: Ki67: 73.8% ± 6.9%; WT-MSC + 131I: Ki67: 76.5% ± 6.7%) (Suppl. Fig. 4D, E).

**DISCUSSION**

In the present study, an emerging gene therapy approach was evaluated where the therapy gene was expressed in the context of eMSC-based protocols - specifically for treatment of metastatic disease. eMSCs are excellent gene delivery vehicles based in part on their relative ease of engineering and expansion in vitro. Importantly, these cells also show a remarkable natural tropism for solid tumors (22, 23). An array of therapy proteins have already been demonstrated to be successfully delivered to tumor environments using eMSC, including interferon-gamma, TRAIL ligands, IL-12, the chemokine CX3CL1, as well as various suicide genes (4, 5, 24, 25). Recent studies have shown that adoptively applied MSC can also efficiently home to tumor metastases opening the door to the potential use of eMSC for treatment of metastatic disease (26-29).

In a recent study, using experimental models of melanoma, breast and hepatoma tumors the authors used an IL-12-based eMSC approach that showed reduced progression of metastases at midstage of development, and even regression at later stages following an extended course of i.v. immunotherapy using IL-12 gene-engineered MSCs (29). In a model of metastatic breast cancer, Zhao *et al.* showed that, human neural stem cells (NSC) engineered to constitutively secrete the suicide gene carboxylesterase, were also able to target tumor metastases in multiple organs including liver, lymph nodes, and lung (27).
The downside of gene therapy using traditional suicide genes is that even though they act through bystander killing, their effects are limited to the cells most proximal to the transgene expressing cells. In contrast, NIS-targeted radioiodine therapy is associated with a higher degree of bystander killing effect that results from the crossfire effect of the β-emitter $^{131}$I and from the radiation-induced biological bystander effect. This therapy concept has been effectively used for the treatment of differentiated thyroid cancer for almost 70 years and still represents one of the most effective systemic anticancer radiotherapies available to the clinician today.

The application of the NIS gene as a combined imaging/therapy gene has been an area of expanded research in various tumor settings over the past few years (13-17). The ability to non-invasively monitor the biodistribution of NIS expression after systemic gene delivery provides an essential extension to the clinical setting. A large number of NIS-compatible radioactive tracers are available including $^{123}$I for scintigraphy/SPECT, and $^{124}$I- and $^{18}$F-TFB (30) for PET imaging. Finally, NIS transgene expression allows the delivery of a robust therapeutic effect through $^{131}$I or $^{188}$Re application (5, 20, 31-36). We have demonstrated the flexibility of this approach in studies using oncolytic viruses and non-viral nanoparticles equipped with the NIS gene in order to facilitate non-invasive in vivo vector biodistribution imaging as well as $^{131}$I-based radiotherapy after systemic application (37, 38).

In previous studies we demonstrated a proof of concept of systemic NIS gene delivery using MSCs as delivery vehicles using subcutaneous xenograft mouse models (4, 5). Systemic application of eMSCs expressing NIS under control of the tumor stroma associated RANTES promoter (RANTES-NIS-MSC) led to a significant radioiodide
accumulation in a subcutaneous liver cancer xenograft model in nude mice resulting in a
dramatic delay of tumor growth and improved survival by therapeutic $^{131}$I application (5).
The potential therapeutic efficacy of MSCs engineered to express NIS using the
RANTES promoter in the treatment of tumor metastases represents a clinically important
open issue. Here a liver metastasis model of colon cancer was used to analyze the
biodistribution of MSC recruitment and NIS-mediated radioiodine accumulation.

By $^{123}$I-gamma-camera and $^{124}$I-PET imaging NIS-eMSCs were shown to specifically
home to liver metastases, and induce RANTES promoter-driven NIS transgene
expression resulting in a tumor-selective radioiodine accumulation. While $^{123}$I
scintigraphic imaging showed a diffusely elevated radioiodine uptake in the hepatic area,
$^{124}$I PET imaging allowed a three-dimensional analysis of NIS-mediated radioiodine
accumulation with higher resolution showing iodide uptake confined to metastatic
nodules. Ex vivo analysis of radioiodine biodistribution confirmed NIS-specific iodine
uptake confined to the liver, which was further validated by quantitative PCR analysis
and immunohistology demonstrating strong NIS and SV40 large T Ag immunoreactivity
strictly confined to metastatic tissue without expression in normal liver tissue or non-
target organs. In our previous study in the subcutaneous liver cancer xenograft mouse
model with normal liver we have also not seen any hepatic NIS and SV40 large T Ag
immunoreactivity after systemic application of RANTES-NIS-MSC, demonstrating that
MSC recruitment into liver metastases is not the result of significant liver pooling of
MSCs (5).

In therapy studies a significant reduction of tumor load in the liver was observed from at
least 90% in the control groups to approx. 60% in the therapy group starting with 40%
tumor load prior to the first $^{131}$I application, which was associated with a significantly
extended life span - which is highly significant considering the aggressive growth of tumor metastases in this model. These findings correlate with markedly reduced proliferation and blood vessel density in the tumors of the therapy group.

In contrast to our study, previous studies of gene therapy of metastatic disease analyzed therapeutic efficacy ex vivo. Zischek et al. showed reduced level of metastases in a pancreatic carcinoma mouse model by ex vivo analysis of tumor growth after treatment with MSCs expressing the HSV-TK suicide gene (6). With the translation from animal to human studies in mind, in the present study we used contrast enhanced MRI to monitor the growth of hepatic colon cancer metastases over time in vivo. MRI is emerging as a powerful method to investigate the potential effects of treatment approaches in mice. The contrast between tumor and normal tissue was enhanced by a liver-specific contrast agent (Primovist®), which allowed an optimal evaluation of treatment effects in hepatic metastases (39).

We are aware that with the mouse model used in this study based on metastases of human colon cancer cells to the liver in athymic nude mice, the influence of the immune system on MSC biodistribution as well as on tumor growth and therapeutic efficacy of MSC-mediated NIS radionuclide therapy cannot be investigated and will be addressed in future studies. MSCs are known to have immunosuppressive functions that may influence therapeutic efficacy. However, previous studies from our group using syngeneic mouse models with well-defined tumor antigens have convincingly demonstrated tumor-selective homing of MSCs without changes of growth dynamics of the tumor after systemic application of control MSCs (6, 9).

CONCLUSION
Non-invasive $^{123}$I-gamma camera and $^{124}$I-PET imaging demonstrated remarkable metastatic selectivity of MSC recruitment and NIS expression driven by the tumor stroma-specific RANTES/CCL5 promoter after systemic MSC application. The tumor stroma-targeted iodide uptake confined to liver metastases was strong enough for a significant reduction of metastases growth monitored in vivo by MRI resulting in significantly improved survival. These data therefore convincingly demonstrate the enormous potential of MSC-mediated NIS gene radionuclide therapy in metastatic cancer.

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DISCLOSURE STATEMENT

No competing financial interests exist.
REFERENCES


Figure 1: Systemic injection of RANTES-NIS-MSCs resulted in enhanced iodide accumulation in livers of mice harbouring colon cancer metastases as shown by $^{123}$I-gamma-camera imaging (A) and small animal $^{124}$I-PET imaging (D, E) two hours after radionuclide injection, which was completely blocked upon treatment with the NIS-
specific inhibitor NaClO₄ (B). I.v. injection of WT-MSCs did not result in enhanced radioiodine accumulation in the liver region (C).
Figure 2: *Ex vivo* $^{123}$I biodistribution studies revealed a hepatic iodide uptake of approx. 6.1 ± 1.1% ID/g, while no significant radiiodine uptake was measured in non-target organs, after pretreatment with perchlorate, or after systemic injection of WT-MSCs. Results are reported as percent of injected dose per organ ±SD.
Figure 3: Following systemic RANTES-NIS-MSC injection, strong NIS-specific immunoreactivity was detected confined to metastatic tissue (A), whereas normal liver tissue did not show NIS-specific immunostaining. No NIS-specific immunoreactivity was detected following systemic injection of WT-MSCs (B). SV40 large T Ag staining demonstrated the presence of RANTES-NIS-MSC (C) and WT-MSC (D) in hepatic colon cancer metastases.
**Figure 4:** Mice received three rounds of RANTES-NIS-MSC or WT-MSC applications followed by $^{131}$I (55.5 MBq) administration. A further control group received saline instead of $^{131}$I. Therapeutic application of $^{131}$I after injection of RANTES-NIS-MSCs resulted in significantly improved survival as compared to the control groups (A). MRI monitoring of metastases growth showed small metastases in the liver with a hepatic tumor load of approx. 40% at day 10 after intrasplenic LS174t cell injection (after three MSC applications and before the first $^{131}$I injection) (B). Exponential tumor growth was observed in the control group (RANTES-NIS-MSC + NaCl) with a tumor load of at least 90% at day 19 (D) in contrast to the therapy group (RANTES-NIS-MSC + $^{131}$I) (C), which revealed delayed metastases growth with a tumor load of approx. 60% at day 19 after intrasplenic tumor cell injection. (M – Metastases, L – Liver, S – Stomach)
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