Interrogating Glioma-Associated Microglia and Macrophage Dynamics Under CSF-1R Therapy with Multitracer In Vivo PET/MRI

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Glioma-associated microglia and macrophages (GAMMs) are key players in creating an immunosuppressive microenvironment. They can be efficiently targeted by inhibiting the colony-stimulating factor 1 receptor (CSF-1R). We applied noninvasive PET/CT and PET/MRI using [18F]-fluoro-2-methoxypyridin-3-yl methyl)-5-((5-methyl-1H-pyrrolo[2,3-b]pyrimidine-3-acetamide (18F-DPA-714) (translocator protein) to understand the role of GAMMs in glioma initiation, monitor in vivo therapy-induced GAMM depletion, and observe GAMM repopulation after drug withdrawal. Methods: C57BL/6 mice (n = 44) orthotopically implanted with syngeneic mouse GL261 glioma cells were treated with differrent regimens using the CSF-1R inhibitor PLX5622 (6-fluoroethoxy)phenyl)-5-(5-fluoro-2-methoxy)pyridin-3-yl)methyl)-5-(5-methyl-1H-pyrrrole[2,3-b]pyridin-2-amine) or vehicle, establishing a preconditioning model and a repopulation model, respectively. The mice underwent longitudinal PET/CT and PET/MRI. Results: The preconditioning model indicated similar tumor growth based on MRI (44.5% ± 24.8%), [18F]-FET PET (18.3% ± 11.3%), and 18F-DPA-714 PET (16% ± 19.0%) volume dynamics in all groups, suggesting that GAMMs are not involved in glioma initiation. The repopulation model showed significantly reduced 18F-DPA-714 uptake (-45.6% ± 18.4%), significantly reduced GAMM infiltration even after repopulation, and a significantly decreased tumor volume (-54.29% ± 8.6%) with repopulation as measured by MRI, supported by a significant reduction in 18F-FET uptake (-50.2% ± 53%). Conclusion: 18F-FET and 18F-DPA-714 PET/MRI allow noninvasive assessment of glioma growth under various regimens of CSF-1R therapy. CSF-1R-mediated modulation of GAMMs may be of high interest as therapy or cotherapy against glioma.

Key Words: glioma; GAMM; 18F-DPA-714; TSPO; CSF-1R; C57BL/6

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The aggressiveness and molecular complexity of glioblastoma multiforme challenges the current standard-care therapy, limiting the median overall survival to 14–16 mo (1). In the last few decades, new immunotherapeutic strategies have been developed for gliomas. Despite some promising results, there are currently no approved immunotherapies that proved to be efficient against glioblastoma multiforme (2). The high heterogeneity of the tumor microenvironment plays an important role in therapy resistance, and glioma-associated myeloid cells, such as glioma-associated microglia and macrophages (GAMMs), monocytes, and myeloid-derived suppressor cells (MDSCs) (including polymorphonuclear and monocytic MDSCs), are key players in the establishment of an immunosuppressive environment that favors glioma immune evasion and progression (3). Moreover, chronically activated resident immune cells exacerbate the inflammatory response, leading to a high state of neuroinflammation while participating in the development of an immunosuppressive tumor microenvironment (4). Therefore, targeting glioma-associated myeloid cells represents an important strategy to develop new glioma microenvironment-targeted therapies. These cell populations are dependent on colony-stimulating factor 1 receptor (CSF-1R) signaling for their repopulation, influencing their ability to modulate GAMMs through a mechanism of depletion and repopulation.

MRI and amino acid PET imaging with methyl-11C-methionine or 18F-fluoroethyltyrosine (18F-FET, amino acid transport) are part of the clinical imaging routine to diagnose and follow up patients with gliomas (6–9). The use of 18F-FET PET has been reported to have a major clinical value in providing important information for tumor delineation and differentiation and for assessment of posttherapeutic modifications and relapses (10). 18F-FET PET is highly specific for glioma tissue but falls short in visualizing the reactive and infiltrating myeloid component of the glioma microenvironment (11). Other radiotracers, including the 18-kDa translocator protein

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(TSPO)–targeting PET tracer N,N-diethyl-2-[4-(2-18F-fluoroethoxy)-phenyl]-5,7-dimethylpyrazolo[1,5-a]pyrimidine-3-acetamide (18F-DPA-714), have been used to visualize the myeloid cell compartment in gliomas (12). 18F-DPA-714 PET has been reported to give information complementary to that from 18F-FET, especially with regard to resident and infiltrating immune cells in preclinical glioma models and in patients (13,14). Because the timing of immunomodulation may be critical for therapy outcome, noninvasive imaging tools are highly useful in the assessment of the glioma microenvironment before, during, and after therapeutic intervention with novel immunomodulatory compounds (15).

Here, we performed a multimodal, dual-tracer imaging study in a syngeneic mouse model of glioma applying 18F-FET and 18F-DPA-714 PET using a CSF-1R inhibitor to investigate the interplay between glioma progression and related immune cell dynamics. Performing a preconditioning study, we aimed to investigate the possible role of GAMMs in glioma initiation. In the repopulation model, we aimed to assess the therapeutic effect of CSF-1R inhibition–induced depletion and subsequent repopulation on GAMM dynamics in an established tumor.

We hypothesized that a GAMM depletion–repopulation approach would be beneficial, resulting in reduced tumor size and decreased neuroinflammation, and that 18F-DPA-714 is a suitable imaging readout for in vivo investigation of GAMM dynamics during the course of CSF-1R inhibitor therapy. Taking advantage of the reversible inhibitory effect of the drug, we were able to noninvasively monitor the dynamics during CSF-1R inhibitor–mediated GAMM depletion and repopulation.

MATERIALS AND METHODS

Cell Culture

Mouse GL261 glioma cells were cultured in T-75 cell culture flasks as an adherent monolayer in Dulbecco modified Eagle medium supplemented with GlutaMAX (Thermo Fisher Scientific), 10% heat-inactivated fetal calf serum, and 1% penicillin/streptomycin at 37°C in a humidified incubator maintained at 5% CO2 before intracranial implantation.

Study Approval

All experiments were conducted in accordance with the German law on the care and use of laboratory animals and approved by the Landesamt für Natur, Umwelt, und Verbraucherschutz of North Rhine–Westphalia and the ARRIVE guidelines (Animal Research: Reporting of In Vivo Experiments) (16).

Study Design

In total, 51 C57BL/6 female mice 8–10 wk old were orthotopically implanted (intrastriatal injection, coordinates in relation to bregma: lateral, −2.0 mm; anterior–posterior, +0.5 mm; dorsal–ventral, −3.0 mm) with 2 × 10⁷ mouse GL261 cells in 2 µL of NaCl, 0.9%. During all experimental procedures, the mice were anesthetized with 1.5%–2% isoflurane (Abbott Animal Health) in 100% O2. After surgery, the animals were weighed daily for at least 3 d and before each imaging session to monitor their health condition (Supplemental Fig. 1; supplemental materials are available at http://jnm.snmjournals.org). Applying different regimens of PLX5622 (6-fluoro-N-((5-fluoro-2-methoxy-pyridin-3-yl)methyl)-5-((5-methyl-1H-pyrrolo[2,3-b]pyridin-3-yl)methyl)pyridin-2-amine), we divided the study into 2 parts, a preconditioning model in which mice were treated with PLX5622 14 d before tumor implantation,
and a repopulation model in which microglial cells were acutely depleted and repopulated. The mice underwent sequential gadolinium-enhanced T1-weighted MRI, as well as $^{18}$F-FET and $^{18}$F-DPA-714 PET/CT, as described previously (17). Full details are available in the supplemental methods and in Figures 1A and 4A.

**MRI Studies**

MRI was conducted for 3-dimensional localization of the glioma and for coregistration with PET/CT images. The mice were anesthetized and the lateral tail vein was cannulated using a 26-gauge catheter. A T1-weighted spin-echo 2-dimensional sequence was acquired in a 1-T nanoScan PET/MRI scanner equipped with an MH20 coil (Mediso Medical Imaging Systems; resolution, 0.27 × 0.27 × 0.9 mm). Gadolinium contrast agent (Gadavist; Bayer Pharmaceutical) was injected via the catheter (50 μmol/kg), and a postgadolinium T1-weighted sequence was acquired.

**PET Studies**

PET images were acquired on a high-resolution small-animal PET scanner (32-module quadHIDAC; Oxford Positron Systems Ltd.) with uniform spatial resolution (<1 mm in full width at half maximum). $^{18}$F-DPA-714 PET images were acquired 60–80 min after intravenous injection of $14.3 \pm 2.6$ MBq of $^{18}$F-DPA-714. $^{18}$F-FET PET images were acquired 20–30 min after intravenous injection of $10.6 \pm 0.6$ MBq of $^{18}$F-FET. The supplemental methods provide further details.

**Volumetric Analysis**

Imaging data were analyzed using the in-house-developed software MEDgical as described previously (17). Briefly, an atlas-based right

![Image](image-url)
samples were analyzed using the Navios of directly labeled monoclonal antibodies (Supplemental Table 2). All based volume dynamics, together with 18F-DPA-714 signal was dis-

Additionally, 18F-DPA-714 uptake already on day 7 in the chronically treated group compared with the nontreated group (Supplemental Fig. 2; Supplemental Tables 3 and 4).

A high density of Iba1-positive (+) cells was detected in preconditioned-and-repopulated mice, as well as in the nontreated group. Chronic treatment reduced the number of Iba-1+ cells, although not as expected, revealing a resistant Iba1 + cell population in both hemi-

Interestingly, we observed an influx of apparently round CSF-1R+, TSPO+ cells within the tumor area (Fig. 3).

**RESULTS**

**18F-DPA-714 PET Imaging Reveals Therapy-Resistant Cells in Chronically Treated Mice**

All the experimental groups in the precon-
ditioning study showed similar gadolinium-

enhanced T1-weighted MRI and 18F-FET–

based volume dynamics, together with 18F-

DPA-714–based volume, indicating increased tumor volume and inflammation over time (Figs. 1B and 2). Tumor volume was signi-


cantly reduced in the chronically treated group compared with the nontreated (Supplemental Fig. 2; Supplemental Tables 3 and 4).

We then assessed the possible effects of acute treatment and subsequent microglial repopulation. In nontreated animals, gadol-
inium-enhanced T1-weighted MRI, 18F-FET PET, and 18F-DPA-

714 PET showed a significant increase in tumor volume and both tracer volumes over time. In the group treated with PLX5622 (6-fluoro-N-(5-fluoro-2-methoxy)pyridin-3-yl)methyl)-5-((5-methyl-
1H-pyrrolo[2,3-b]pyridin-2-amine), gadolinium-

enhanced T1-weighted MRI and 18F-FET–derived volumes significantly increased between days 7 and 21 whereas 18F-DPA-

714 PET–derived volume significantly increased between days 7 and 14 (Figs. 4B and 5). A therapy effect was observed on day 21,
when repopulated animals showed significantly reduced tumor volumes, 18F-FET PET-derived volumes, and 18F-DPA-714 PET-derived volumes compared with nontreated animals (Supplemental Table 5).

In the nontreated group, 18F-FET tumor mean/background mean significantly increased between days 7 and 21, whereas in the PLX5622-plus-repopulation group it increased between days 14 and 21. Similarly, 18F-FET tumor maximum/background mean was increased in the nontreated group but was significantly reduced in repopulated animals on day 21 (Supplemental Fig. 4; Supplemental Table 6).

In-depth analyses already showed a statistically significant positive correlation between MRI-based tumor volume and 18F-FET uptake on days 14 and 21 (Supplemental Fig. 7).

**GAMMs Are Significantly Affected by CSF-1R Inhibition, with Persistent Reduction in CSF-1R and TSPO Expression After Drug Withdrawal**

PLX5622 treatment successfully depleted most Iba1+ cells in glioma-bearing mice compared with the nontreated group ($P \leq 0.05$), in both the ipsilateral and the contralateral hemispheres, in line with TSPO PET. The remaining Iba1+ cells were at the periphery of the glioma or within the tumor borders, displaying an ameboid-like morphology indicative of an active state. After 1 wk of repopulation, the number of Iba-1+ cells on the contralateral side was significantly increased in the repopulation group compared with the PLX5622 group ($P \leq 0.05$) (Supplemental Fig. 5).

Similarly, CD68 expression was analyzed as a marker for GAMMs and specifically for macrophage-mediated immune suppression. A large number of CD68+ cells was detected infiltrating the glioma tissue in nontreated animals. The number of CD68+ cells was significantly reduced with PLX5622 treatment and remained significantly reduced after repopulation as shown by quantification (Supplemental Fig. 6).

CSF-1R+ tumor cells, Iba1+ microglial cells at the periphery and infiltrating the glioma, and CSF-1R+, Iba1+ cells within the tumor mass were detected in nontreated animals. PLX5622 treatment reduced CSF-1R expression, with few CSF-1R+ cells present at the border of the glioma. After repopulation, CSF-1R signal remained reduced and CSF-1R+ cells were visible at the periphery of the glioma (Fig. 6).

Similarly, TSPO+ cells and Iba1+ cells, as well as TSPO+, Iba1+ cells, surrounded and infiltrated the glioma tissue in the nontreated group. After CSF-1R inhibition, TSPO signal was detectable mostly at the border of the tumor mass, in line with the imaging results, together with a few Iba1+ and TSPO+Iba1+ cells. After repopulation and in accordance with the imaging results, the reduction in TSPO signal remained stable, and TSPO+ cells were visible at the periphery of the glioma whereas cells that were Iba1+, TSPO-negative and Iba1+, TSPO+ were detectable within the glioma tissue (Fig. 7). Interestingly, the depletion of Iba1+ cells seemed to promote the infiltration of as-yet-uncharacterized CSF-1R+, TSPO+ cells (Figs. 6 and 7).

**CSF-1R Inhibition Shows Immune-Modulatory Effects on Glioma-Associated Myeloid Tissue–Derived Cells**

To further evaluate therapy-induced changes in the tumor microenvironment and to characterize the phenotype of infiltrating cells, multiparametric flow cytometry was performed (Supplemental Fig. 8). PLX5622 treatment reduced the frequency of CD45+ tumor-infiltrating leukocytes compared with the nontreated group (15.2% vs. 22.3%, respectively), in which the frequency was slightly increased after repopulation (19.1%). The same results were found for GAMMs, confirming the histologic results. Comparable amounts of total MDSCs were detected in the PLX5622-treated and the nontreated groups (CD11b+, Gr 1+: 5.1% vs. 3.6%), which significantly decreased after repopulation (CD11b+, Gr 1+: 0.7%). Moreover, PLX5622 treatment produced a difference in the MDSC phenotype and affected the expression of different M2 activation markers, such as TSPO and major histocompatibility complex class II. The supplemental appendix provides additional information.

**DISCUSSION**

This study aimed to assess the suitability of a multitracer PET/MRI combination to investigate the effects of microglia-depleting immunotherapy in the tumor microenvironment in a syngeneic mouse glioma model. Using different treatment regimens, we demonstrated that the dual-tracer combination of 18F-FET and 18F-DPA-714 PET in conjunction with MRI allows monitoring of therapy-induced changes in glioma progression and GAMM dynamics in the tumor microenvironment. In contrast to 18F-FET...
Finally, TSPO PET using 18F-DPA-714 gives information complementary to the imaging marker 18F-FET, supporting the efficacy of CSF-1R PET in detecting glioma progression and neuroinflammation. Furthermore, TSPO PET has great potential for the characterization and imaging of the glioma microenvironment, as shown in the first-in-patients study by Zinnhardt et al. (14).

The importance of choosing the proper therapeutic window for GAMM modulation is crucial, and TSPO PET may serve this purpose (23). In this regard, a short-term (1 wk) CSF-1R inhibition with subsequent brain repopulation during glioma progression led to ablation of microglia cells and GAMMs before and during glioma development showing that the inhibition of these cell populations before glioma initiation does not influence glioma formation or the inflammatory response. Ex vivo characterization revealed the presence of a potentially resistant Iba1+ cell population as a possible major cellular source of TSPO expression, particularly in chronically treated animals. These findings were supported by in-depth histologic analyses that showed a persistent reduction in TSPO and CSF-1R signals coming from the glioma tissue, whereas a high number of TSPO+ and CSF-1R+ cells were infiltrating the glioma microenvironment. The same holds true for the preconditioned-and-repopulated group characterized by the presence of a high number of GAMMs and microglia cells positive for all the markers infiltrating the glioma tissue. These results might explain the increased 18F-DPA-714 uptake over time, highlighting the importance of TSPO PET in detecting glioma-associated cell infiltration.

Whether the observed resistant cell population is pro- or antitumorigenic has yet to be clarified. However, it partially expressed TSPO, supporting the efficacy of TSPO PET in monitoring specific glioma-associated inflammation. Furthermore, TSPO PET has great potential for the characterization and imaging of the glioma-associated immunosuppressive tumor microenvironment, as shown in the first-in-patients study by Zinnhardt et al. (14).

The role of the CSF-1R/CSF-1 axis has been studied in many cancer types, because tumor cells secrete CSF-1 to attract CSF-1R+ myeloid-derived cells to the tumor microenvironment to sustain tumor progression. Therefore, the expression levels of CSF-1R and circulating ligand are regarded as biomarkers for tumor progression, treatment failure, and poor prognosis (19). Different CSF-1R inhibitors have been tested in preclinical studies and clinical trials but failed to show substantial efficacy because of acquired resistance to CSF-1R therapy by the glioma microenvironment (20).

TSPO is reported to be upregulated by resident (microglia, astrocytes) and peripheral (monocytes, lymphocytes, neutrophils) immune cells during inflammatory conditions (21). In glioma tissue, TSPO is expressed by glioma cells, GAMMs, and endothelial cells (22). Our group recently described the suitability of TSPO PET using 18F-DPA-714 to monitor treatment outcomes in a preclinical glioma model, by identifying areas of myeloid cell infiltration within the glioma microenvironment (17). Our results show the efficacy of CSF-1R inhibition for the blockade of GAMM proliferation and that the combination of 18F-FET and 18F-DPA-714 PET can be used to monitor CSF-1R therapy–induced changes in the glioma microenvironment. The CSF-1R inhibitor PLX5622 proves to have a disease-changing effect, reducing glioma progression and neuroinflammation, particularly when administered for a short period during established glioma growth.

We report data on ablation of microglia cells and GAMMs before and during glioma development showing that the inhibition of these cell populations before glioma initiation does not influence glioma formation or the inflammatory response. Ex vivo characterization revealed the presence of a potentially resistant Iba1+ cell population as a possible major cellular source of TSPO expression, particularly in chronically treated animals. These findings were supported by in-depth histologic analyses that showed a persistent reduction in TSPO and CSF-1R signals coming from the glioma tissue, whereas a high number of TSPO+ and CSF-1R+ cells were infiltrating the glioma microenvironment. The same holds true for the preconditioned-and-repopulated group characterized by the presence of a high number of GAMMs and microglia cells positive for all the markers infiltrating the glioma tissue. These results might explain the increased 18F-DPA-714 uptake over time, highlighting the importance of TSPO PET in detecting glioma-associated cell infiltration.

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The importance of choosing the proper therapeutic window for GAMM modulation is crucial, and TSPO PET may serve this purpose (23). In this regard, a short-term (1 wk) CSF-1R inhibition with subsequent brain repopulation during glioma progression led
to a significant reduction in tumor volume as quantified by both gadolinium-enhanced MRI and $^{18}$F-FET PET. In accordance with the literature, CSF-1R inhibition significantly reduced the number of Iba1+ cells in the brain (24). However, tumor cells also express TSPO, and the volumetric analysis showed increased $^{18}$F-DPA-714 PET volumes on day 14 at the same level as in the nontreated group because of glioma progression, which may hide possible immunologically induced changes. Interestingly, after 7 d of brain repopulation, the $^{18}$F-DPA-714 PET signal remained stable and a significant reduction in tracer volume was detected compared with the control group. This finding may indicate that repopulation could modulate the immunosuppressive function toward an antitumor phenotype, highlighting the importance of targeting GAMMs.

Validation of TSPO PET tracers in clinical settings is necessary to improve the understanding of glioma-associated inflammation and therapy resistance mechanisms.

CONCLUSION

$^{18}$F-DPA-714 may be a useful imaging biomarker for longitudinal therapy monitoring and assessment of the glioma-associated inflammatory microenvironment, as well as for patient stratification. CSF-1R–targeting intervention and determination of the ideal treatment window for CSF-1R inhibitors may define a promising complementary therapy strategy in glioma.

DISCLOSURE

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The CSF-1R therapy–induced effects on the immune component were further investigated by multiparametric flow cytometry analyses. Overall, the analyses indicated a strong effect of CSF-1R immune modulation on the MDSC population. The results suggest that the infiltrating component in the absence of Iba1+ cells might be represented by polymorphonuclear MDSCs. Moreover, PLX5622 effects resulted in the modulation of major histocompatibility complex class II and TSPO expression on GAMMs and MDSCs, in line with the reduction in $^{18}$F-DPA-714 PET volumes. This finding may indicate that repopulation could modulate the immunosuppressive function toward an antitumor phenotype, highlighting the importance of targeting GAMMs.

As reported, single-agent therapy with CSF-1R inhibitors has demonstrated only modest results in glioblastoma clinical trials, showing no significant improvement in the progression-free survival of patients (27). Currently, other ongoing studies are combining CSF-1R therapy and immune-checkpoint inhibitors in different types of tumors (28). One pilot study evaluating the TSPO PET tracer $^{11}$C-PBR28 in patients with primary glioblastoma multiforme or melanoma brain metastasis, treated with chemoradiation or immunotherapy, was completed recently; the results are to be determined (NCT02431572) (29).
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**KEY POINTS**

**QUESTION: Is 18F-DPA-714 PET suitable to decipher glioma-associated immune cell dynamics and CSF-1R therapy outcomes?**

**PERTINENT FINDINGS:** 18F-DPA-714 PET provided complementary information to 18F-FET PET on glioma heterogeneity, allowed the imaging of immune cell dynamics, supported the assessment of brain target engagement, and elucidated CSF-1R inhibition–induced effects in vivo.

**IMPLICATIONS FOR PATIENT CARE:** 18F-DPA-714 PET represents a promising tool for noninvasive imaging of therapeutic modulations of the immunosuppressive glioma microenvironment.

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