# [$^{18}$F]-*N*-{4-[(4,5-Dichloro-2-fluorophenyl)amino]quinazoline-6-yl}-dimethylamine-butylamide

## [$^{18}$F]ML04

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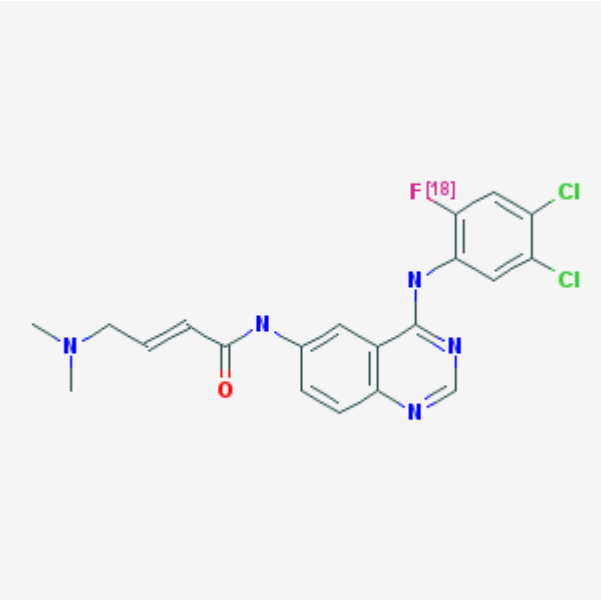
| **Chemical name:** | [$^{18}$F]-*N*-{4-[(4,5-Dichloro-2-fluorophenyl)amino]quinazoline-6-yl}-dimethylamine-butylamide |
| **Abbreviated name:** | [$^{18}$F]ML04 |
| **Synonym:** | [$^{18}$F]TKS040 |
| **Agent Category:** | Ligand (irreversible inhibitor) |
| **Target:** | Epidermal growth factor receptor tyrosine kinase (EGFR-TK) |
| **Target Category:** | Enzyme |
| **Method of detection:** | Positron emission tomography (PET) |
| **Source of signal / contrast:** | $^{18}$F |
| **Activation:** | No |
| **Studies:** | • *In vitro*<br>• Rodents |

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## Background

[PubMed]

The epidermal growth factor receptor (EGFR) mediates its activity through an associated intracellular tyrosine kinase (TK) that controls the signal transduction pathway(s) responsible for the stimulation of cell processes such as growth, proliferation, and motility necessary for the progression of cancers (1). Although constitutive activation of the EGFR or a mutated TK are known to promote the development of various cancers, a characteristic feature of some malignantly transformed cells is the overexpression of EGFR or the receptor ligands that result in a constant activation of the receptor (2). In addition, an overactive TK, due to EGFR activation, a gene mutation, or overexpression, may also provide a stimulus for a ligand-free, and unregulated, initiation of the signal transduction pathway(s). Because of their role in the development of the oncogenic phenotype, the EGFR and its associated TK are respectively targeted with antibodies and small molecule inhibitors, respectively0 for the treatment of various cancers (3). Several of these agents are under evaluation in clinical trials approved by the United States Food and Drug Administration. The EGFR-TK inhibitors bind, reversibly or irreversibly, to the intracellular ATP binding site of the enzyme, prevent autophosphorylation of the receptor, and inhibit the signal transduction pathway(s) responsible for initiation of the biological processes mediated by the EGFR (3, 4).

Determination of the EGFR content of cancerous tumors with noninvasive imaging techniques could help monitor cancer patients who are likely to benefit from an anti-EGFR or anti-TK therapy and perhaps predict the treatment prognosis for patients. The use of labeled reversible EGFR-TK inhibitors with positron emission tomography (PET) for the imaging of EGFR was evaluated earlier (5, 6), but these imaging agents were determined to be unsuitable for this purpose because of the high intracellular ATP content that resulted in rapid clearance of the label from the cells, rendering the compounds ineffective for PET imaging of tumors. To alleviate the limitations observed with the reversible TK inhibitor PET probes, investigators developed and evaluated the use of nuclide-labeled irreversible TK inhibitors for the imaging of EGFR (7, 8). Mishani et al. reported that derivatives of the 4-dimethylamino-but-2-enoic acid [4-(phenylamino)-quinazoline-6-yl]-amide group had a high chemical and biological stability as well as high specificity as irreversible inhibitors of the EGFR-TK (9). On the basis of these observations, Abourbeh et al. developed and characterized an $^{18}$F-labeled PET imaging compound, [$^{18}$F]ML04 (also known as [$^{18}$F]TKS040), for EGFR-TK inhibition under *in vitro* conditions (10). Abourbeh et al. also studied the biodistribution of this radiochemical in mice bearing EGFR-overexpressing xenograft tumors.

## Synthesis

[PubMed]

ML04 was synthesized as described by Mishani et al. (9). The $^{18}$F labeling of ML04 to yield [$^{18}$F]ML04 was performed as detailed elsewhere (10). The average radiochemical yield and purity of the tracer was 14% and 98% ($n$ = 10 reactions), respectively, as determined with high-performance liquid chromatography. The total time of radiosynthesis, including purification and formulation, was 4 h. The specific activity of [$^{18}$F]ML04 was reported to be 6.66 TBq/mmol (1,800 Ci/mmol).

## *In Vitro* Studies: Testing in Cells and Tissues

[PubMed]

Abourbeh et al. investigated the inhibition of EGFR autophosphorylation by ML04 with human epithelial carcinoma A431 cells (high EGFR expression), human breast carcinoma MDA-MB-468 cells (EGFR overexpression), human lung carcinoma PC10 cells (EGFR overexpression), and human non-small cell lung carcinoma (NSCLC) NIH-H1975 cells (mutant EGFR expression) (10) as described by Mishani et al. (9). The cells were exposed to the inhibitor for 1 h as described, and cell lysates were prepared for analysis. The 50% inhibitory concentration ($IC_{50}$) values of the radiolabel for EGFR autophosphorylation of the A431, MDA-MB-468, PC10, and NIH-H1975 NSCLC cells was reported to be 4–10, 1–5, 10–50, and 25 nM, respectively. Another set of the respective cells was exposed to ML04 for 1 h, and the cells were washed with phosphate-buffered saline. The cells were then incubated in growth medium for 8 h, and the $IC_{50}$ values of EGFR autophosphorylation were determined. The $IC_{50}$ values of the radiolabel were reported to be similar to those given above (10). With results obtained from this study, the investigators concluded that ML04 is an irreversible inhibitor of the EGFR in the respective cells.

In another study, the investigators showed that [$^{18}$F]-ML04 could be used to determine the number of EGFR on A431 cells (10). They reported that on average there were 1.56 $\pm$ 0.36 $\times$ $10^6$ EGFR per cell ($n$ = 4 determinations), which was close to the numbers reported earlier (1–2 $\times$ $10^6$ EGFR per cell) by other investigators (10). However, these investigators cautioned that [$^{18}$F]ML04 was an appropriate tool to determine the number of EGFR on cells only if used at carefully adjusted concentrations.

## Animal Studies

### Rodents

[PubMed]

The biodistribution of [$^{18}$F]ML04 was investigated in Nude-Hsd athymic nude-nu mice bearing U87MG.wt EGFR tumors (a human glioma cell line that overexpresses the wild-type EGFR (11)) (10). Mice bearing U187MG tumors, an EGFR-negative human glioma cell line, were used as controls for this study. [$^{18}$F]ML04 was injected into the mice through the lateral tail vein, and blood was drawn from the animals at predetermined time points. The animals were euthanized to excise selected organs to determine the

amount of accumulated radioactivity in each organ as a percent of injected dose per gram of tissue (% ID/g). Preliminary experiments showed that the optimal uptake of radioactivity in the various organs and tumors was obtained at 3 h after treatment with the radiotracer. These studies also demonstrated that the two vehicles used (5–10% ethanol/saline ($n$ = 12 animals) and IntraLipid ($n$ = 37 animals)) to administer [$^{18}$F]ML04 to the animals behaved similarly, and no statistical difference was noted in the biodistribution of radioactivity with either vehicle.

Abourbeh et al. compared the uptake of [$^{18}$F]ML04 by U87MG.wt EGFR cell xenograft tumors ($n$ = 15–47 animals/time point) with the uptake by control U138MG xenograft tumors ($n$ = 5–14 mice/time point) (10). Animals in the two groups were injected with [$^{18}$F]ML04 as before and euthanized 120, 180, and 240 min after treatment. The tumors and organs of interest were excised from the animals, and the accumulated radioactivity was determined. Compared with the control tumors (accumulated radioactivity was ~0.65% ID/g at all time points), a significantly higher (statistical $P$ value not reported) amount of radioactivity was reported to have accumulated in the U87MG.wt EGFR tumors at all time points (~1.1% ID/g at 120 min and ~0.8% ID/g at 240 min). Although all other organs from these animals showed a decrease in accumulated label from 120–240 min, the fact that the U138MG tumors maintained a constant level of radioactivity suggested that these tumors had a nondisposable blood pool due to a more necrotic nature compared with the U87MG.wt EGFR tumors.

To determine the target specificity of [$^{18}$F]ML04, Abourbeh et al. performed blocking studies using an excess of unlabelled ML04 (10). Tumor-bearing mice ($n$ = 12 animals) were injected with 5–8 mg ML04/kg body weight 1 h prior to treatment with the tracer. For this study, control animals ($n$ = 54 mice) received only [$^{18}$F]ML04 at the same time as animals with a prior treatment of unlabeled ML04. The animals were euthanized 3 h after injection of the label, and radioactivity accumulated in the various organs, including tumors, was determined as before. An examination of the amount of accumulated radioactivity in various tissues of the two groups of animals revealed that, in general, animals with the unlabeled ML04 treatment had a higher amount (although not statistically significant) of accumulated radioactivity compared with the control animals. However, the tumor/tissue label ratios of the blocked animals were significantly lower ($P$ value not reported) compared with the control animals, indicating a lower accumulation of radioactivity in tumors compared to the other tissues.

With results obtained from the respective biodistribution studies, the investigators concluded that the uptake of radioactivity in the tumors may not be entirely caused by EGFR binding (10). They also concluded that the distribution of [$^{18}$F]ML04 in the various tissues was probably flow-dependent, and a possible change in chemical structure or the route of administration could help improve tissue dispersal of the radioactivity.

## Other Non-Primate Mammals

[PubMed]

No references are currently available.

### Non-Human Primates

[PubMed]

No references are currently available.

## Human Studies

[PubMed]

No references are currently available.

## Supplemental Information

[Disclaimer]

## References

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