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# Antiepidermal growth factor variant III scFv fragment: effect of radioiodination method on tumor targeting and normal tissue clearance

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**Abstract**

**Introduction:** MR1-1 is a single-chain Fv (scFv) fragment that binds with high affinity to epidermal growth factor receptor variant III, which is overexpressed on gliomas and other tumors but is not present on normal tissues. The objective of this study was to evaluate four different methods for labeling MR1-1 scFv that had been previously investigated for the radioiodinating of an intact anti-epidermal growth factor receptor variant III (anti-EGFRvIII) monoclonal antibody (mAb) L8A4.

**Methods:** The MR1-1 scFv was labeled with $^{125}I/^{131}I$ using the Iodogen method, and was also radiohalogenated with acylation agents bearing substituents that were positively charged—*N*-succinimidyl-3-[*I]iodo-5-pyridine carboxylate and *N*-succinimidyl-4-guanidinomethyl-3-[*I]iodobenzoate ([*I]SGMIB)—and negatively charged—*N*-succinimidyl-3-[*I]iodo-4-phosphonomethylbenzoate ([*I]SIPMB). In vitro internalization assays were performed with the U87MGΔEGFR cell line, and the tissue distribution of the radioiodinated scFv fragments was evaluated in athymic mice bearing subcutaneous U87MGΔEGFR xenografts.

**Results and Conclusion:** As seen previously with the anti-EGFRvIII IgG mAb, retention of radioiodine activity in U87MGΔEGFR cells in the internalization assay was labeling method dependent, with SGMIB and SIPMB yielding the most prolonged retention. However, unlike the case with the intact mAb, the results of the internalization assays were not predictive of in vivo tumor localization capacity of the labeled scFv. Renal activity was dependent on the nature of the labeling method. With MR1-1 labeled using SIPMB, kidney uptake was highest and most prolonged; catabolism studies indicated that this uptake primarily was in the form of ε-N-3-[*I]iodo-4-phosphonomethylbenzoyl lysine.



*Keywords:* Radioiodination; Monoclonal antibody; EGFRvIII; Growth factor receptor

## 1. Introduction

The epidermal growth factor receptor variant III (EGFRvIII) is a mutant form of EGFR and is expressed in gliomas and in breast, lung and ovarian carcinomas. Importantly for targeting, EGFRvIII is not expressed detectably by normal tissues, including those with high levels of wild-type EGFR expression [1]. Monoclonal antibodies (mAbs) that bind to EGFRvIII are known to undergo rapid internalization and lysosomal degradation. When radioiodinated directly, these mAbs exhibited only modest retention of radioactivity in tumor due to rapid egress of labeled catabolites, principally monoiodotyrosine [2].

Alternative labeling methods have been designed to surmount this problem. Earlier, we have reported on the preparation and evaluation of L8A4, a murine anti-EGFRvIII mAb, radiohalogenated with acylation agents bearing substituents that were positively charged [2–5]—*N*-succinimidyl-3-[*I]iodo-5-pyridine carboxylate ([*I]SIPC) and *N*-succinimidyl-4-guanidinomethyl-3-[*I]iodobenzoate ([*I]SGMIB) — and negatively charged [6,7]—*N*-succinimidyl-3-[*I]iodo-4-phosphonomethylbenzoate ([*I]SIPMB).

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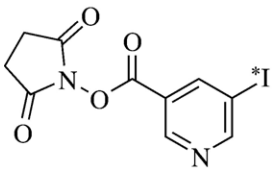
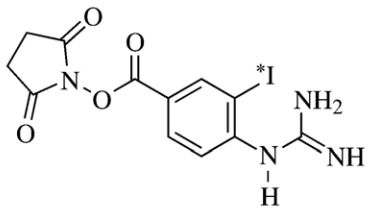
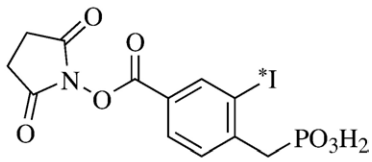


[*I]SIPC



[*I]SGMIB



[*I]SIPMB

These active esters are structurally related to the prototypical labeling agent *N*-succinimidyl-3-iodobenzoate (SIB) [8–10] and are conjugated to the mAb *via* ε-amino groups on constituent lysine residues. At lysosomal pH, labeled catabolites arising from the lysosomal degradation of anti-EGFRvIII mAb L8A4 labeled using [*I]SIPC and [*I]SGMIB primarily were positively charged, whereas those from L8A4 labeled using [*I]SIPMB were negatively charged. Importantly, use of these acylation agents for labeling L8A4 resulted in significant improvements in intracellular retention of radioiodine in EGFRvIII-expressing tumor cells in vitro and in tumor xenografts compared with mAb labeled using the Iodogen or SIB methods.

Because of their more rapid tumor uptake and normal tissue clearance, single-chain Fv (scFv) fragments are an attractive alternative to whole immunoglobulins, particularly for imaging and therapy applications involving short half-life radiohalogens such as $^{18}$F, $^{123}$I and $^{211}$At. With this goal in mind, an anti-EGFRvIII scFv, MR1, was developed [11], and subsequently, combinatorial variation of CDR3, followed by phage display, was utilized to identify MR1-1, a mutant with a 15-fold higher affinity compared with the original scFv [12]. Paired-label comparisons performed with scFv labeled using Iodogen demonstrated that the higher affinity of MR1-1 resulted in a greater degree of internalization in vitro and tumor retention in vivo compared with MR1.

One approach for improving the targeting characteristics of scFv molecules is to optimize the radiolabeling method. As noted above, the nature of the acylation agent utilized for the radiohalogenation of an intact anti-EGFRvIII mAb had a profound effect on the retention of radioiodine in EGFRvIII-expressing tumor cells. The purpose of the current study was to determine whether these acylation agents bearing charged substituents resulted in enhanced targeting with MR1-1 scFv. Unlike intact mAbs, scFv fragments are sufficiently small to undergo glomerular filtration, and a major limitation of scFv molecules as radionuclide carriers is the very high accumulation of radioactivity in the kidney [13,14], which is thought to be mediated in part by the negative charge on the kidney [15,16]. Because SIPC, SGMIB and SIPMB all result in the coupling of a charged prosthetic group to proteins, we were particularly interested in determining whether this variable could be utilized to reduce the renal accumulation of radiolabeled scFv molecules.

## 2. Materials and methods

### 2.1. General

All chemicals were purchased from Aldrich unless otherwise noted. Sodium [$^{125}$I]iodide and sodium [$^{131}$I]iodide, with specific activities of 1200 and 2200 Ci/mmol, respectively, were obtained from Perkin-Elmer Life Sciences.

High-pressure liquid chromatography was performed using a Beckman System Gold HPLC equipped with a Model 126 programmable solvent module, a Model 166 NM variable wavelength detector, a Model 170 radioisotope detector and a Beckman System Gold remote interface module SS420X, using a 32-karat software. A Waters μ Bondapak C18 (10 μm, 3.9×300 mm) column was used for reversed-phase chromatography. Normal-phase HPLC was done using a 4.6×250-mm Partisil (10 μm) silica column (Alltech, Deerfield, IL). Size-exclusion HPLC was performed using a 7.5×600-mm Tosohaas TSK gel G3000 SW (10 μm) gel filtration column (Montgomeryville, PA) eluted with PBS at 1 ml/min. Radio-TLC was analyzed initially using a System 200 Imaging Scanner (BioScan, Washington, DC), after which, the TLC plate was cut into strips that were counted on an automated gamma counter (LKB 1282, Wallac, Finland). Phosphor-imaging (autoradiography) of electrophoresis gels was performed on a Cyclone Phosphor Scanner (Packard Bioscience ) and analyzed using the Optiquant 4.00 software.

### 2.2. Anti-EGFRvIII single-chain fragment MR1-1

MR1-1 is an scFv fragment that recognizes an epitope within the EGFRvIII-specific sequence LEEKKG-NYVVTDHSGGK [17]. The MR1-1 scFv was produced by combinatorial variation of the CDR3 domains of $V_H$ and $V_L$ followed by phage display, and it has an affinity of $1.5\times10^{-9}$ M for the extracellular domain of EGFRvIII [12]. Details of the production, purification and characterization of MR1-1 are described elsewhere [12,17]. Prior to radiolabeling, the 26-kDa scFv was >98% pure as determined by size-exclusion HPLC.

### 2.3. Preparation of radioiodinated N-succinimidyl esters

The protein acylation agents [$^{125}$I]SIPC, [$^{125,131}$I]SGMIB and [$^{125,131}$I]SIPMB were prepared using previously reported procedures [2,4,6]. Briefly, to 1–2 mCi of radioiodine in 1–3 μl NaOH in a one-half dram vial was added 0.4 mg of *N*-chlorosuccinimide in 10 μl AcOH, to which was added