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SGM-101: An innovative near-infrared dye-antibody conjugate that targets CEA for fluorescence-guided surgery

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ABSTRACT

Purpose: Fluorescence-guided surgery (FGS) provides surgeons with new opportunities to improve real-time cancer nodule detection and tumor margin visualization. Currently, the most important challenge in this field is the development of fluorescent dyes that specifically target tumors. We developed, characterized and evaluated SGM-101, an innovative antibody-dye conjugate in which the fluorochrome BM104, which has an absorbance band centered at 700 nm, is coupled to a chimeric monoclonal antibody (mAb) against carcinoembryonic antigen (CEA).
Methods: The dye to mAb ratio, binding to CEA and photobleaching of SGM-101 were determined. FGS was performed and results analyzed using different mouse models of human digestive tumors.
Results: SGM-101 allowed the detection of tumor nodules in three different colon cancer models: LS174T human colorectal adenocarcinoma cell-induced peritoneal carcinomatosis (PC) and liver metastases, and orthotopic grafts of HT29 human colorectal adenocarcinoma cells. In the PC model, submillimeter-sized nodules were detected during SGM-101-based FGS and SGM-101 predictive positive values ranged from 99.04% to 90.24% for tumor nodules >10 mg and nodules <1 mg, respectively. Similarly, in the orthotopic model of pancreatic cancer using BxPC3 (pancreas adenocarcinoma) cells, SGM-101 could clearly delineate tumors *in vivo* with a tumor-to-background ratio of 3.5, and penetrated in tumor nodules, as demonstrated by histological analysis. Free BM105 dye (BM104 with an activated ester for conjugation to the antibody) and an irrelevant conjugate did not induce any NIR fluorescence.
Conclusion: These preclinical data indicate that SGM-101 is an attractive candidate for FGS of CEA-expressing tumors and is currently assessed in clinical trials.

1. Introduction

Digestive cancers are the most frequent and deadly tumors in both men and women (304 000 new cases and 153 000 deaths estimated for 2016 in the USA) [1]. Among them, colorectal cancer is the third most common cancer and leading cause of death in the USA [1]. In approximately 15% of patients with colorectal cancer, peritoneal carcinomatosis is already present at the time of diagnosis, and in 40% of patients, the disease is estimated to progress to peritoneal metastases after the initial diagnosis [2,3]. The extent of

Abbreviations: CEA, Carcinoembryonic antigen; mAb, monoclonal antibody; IPD, immunophotodetection; FGS, fluorescence image-guided surgery; NIR, near infrared; SPR, surface plasmon resonance; TBR, tumor to background ratio.

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peritoneal disease is often underestimated due to the low sensitivity and specificity of the current diagnostic tools (CT, MRI) in detecting small-size tumor nodules [4]. Currently, the most reliable diagnosis is obtained by direct observation through laparotomy or laparoscopy.

Until recently, peritoneal carcinomatosis was considered a terminal condition by most oncologists due to its very poor prognosis and was treated only with palliative care. In recent years, Sugarbaker et al. have introduced a new therapeutic approach that combines cytoreductive surgery, to remove all visible tumor deposits from the peritoneal surface, and hyperthermic intraperitoneal chemoperfusion, to target the microscopic residual disease [5,6]. This treatment strategy has drastically improved the primary and secondary prognosis of peritoneal carcinomatosis. The degree of macroscopic tumor resection is recorded using the completeness of cytoreduction (CCR) score (CCR-0 corresponds to absence of residual macroscopic peritoneal disease). Attaining a CCR-0 resection can significantly improve the 5-year survival rates of patients with colorectal cancer [7]. Therefore, powerful intraoperative imaging techniques are urgently needed to help achieving CCR-0 resection and for the accurate evaluation of tumor extension [8].

Pancreatic cancer is less frequent (around 53 000 new cases expected in the USA in 2016), but its mortality rate is very close to the incidence rate (42 000 expected deaths) [1]. New techniques to improve the current surgery outcomes should be developed, particularly to avoid positive resection margins that occur in 30% of patients [9].

Fluorescence imaging could provide surgeons with solutions to improve tumor resection thanks to the recent development of fluorescence-guided surgery (FGS) (see Refs. [10] and [11] for review). FGS becomes feasible because there are now several commercially available fluorescence image-guided surgery camera systems [11]. However, the currently approved dyes (fluorescein, methylene blue, ICG, IRD800CW) are non-targeted and/or are not in the near infrared (NIR) range, which is the optimum for *in vivo* applications [12]. High-performance NIR fluorescent targeted agents are required to provide surgeons with optimal guidance [13]. Monoclonal antibodies (mAbs) appear to be the best option to target NIR dyes [14]. Although it took more than twenty years to move from mice [15,16] to men and from pioneers' clinical work [17] to clinical studies, the translation of immunophotodetection (IPD; i.e., the use of dyes covalently linked to specific mAbs for the intra-operative detection of tumors) into the clinic seems now very close [18–21].

In the present study and as a first step toward clinical applications, we present the development and characterization of SGM-101, an innovative NIR reagent in which an original dye, BM104, is coupled to an anti-carcinoembryonic antigen (CEA) chimerized mAb. The good results obtained in different murine models of digestive cancers (orthotopic tumors, peritoneal carcinomatosis and hepatic metastases) and the results of extensive toxicological studies warrant a rapid clinical translation.

2. Materials and methods

2.1. Preparation and characterization of SGM-101 and control antibody-dye conjugates

The anti-CEA chimeric mAb SGM-ch511 was produced on a large scale (0.8 g/l) using a clinical compatible subclone of the CHO Protein-Free cell line (European Collection of Authenticated Cell Cultures, cat number 00102307) derived at Vivalis, France. Aliquots of 1 mg of SGM-ch511 in phosphate/bicarbonate buffer pH 9.3 were mixed with BM105 (BM104 with an activated ester for conjugation to the antibody) or Alexa-Fluor 680 (AF680) dissolved in dimethylformamide (final concentration: 2.25 mg/ml) or with IRDye800-CW in bicarbonate buffer pH 8.5, at the indicated initial molar ratio. After agitation at 50 rpm at room temperature in the dark for 45 min, the dye-mAb conjugates were separated from free dye on PD-10 columns (GE Healthcare) equilibrated in elution buffer (10 mM KH_2PO_4, 10 mM Na_3Citrate, 300 mM Arginine, 0.02% (w/w) Tween-20, pH 6.0). The dye/mAb molar ratios were calculated using the mAb and dye molarities determined from optical density (OD) reading at 280 nm, 680 nm and 800 nm for AF680 ($\varepsilon = 183\,000\ cm^{-1}.M^{-1}$) and IRDye800-CW ($\varepsilon = 240\,000\ cm^{-1}.M^{-1}$) conjugates, respectively. For BM105 conjugates, the samples' absorbance was measured at 280 and 685 nm using a spectrophotometer (Jenway 7315, Bibby Scientific France) and then the labeling ratios were calculated using the following formula: Dye/protein $= (M_{dye})/((A_{280}/(0.3 \times \varepsilon_{protein})/MW_{protein}))$, where the molar extinction coefficient of the protein ($\varepsilon_{protein}$) was 1.5 $M^{-1}\ cm^{-1}$, the molecular weight of the protein ($MW_{protein}$) was 150 000 g mol^{-1}. The molarity of the dye (M_{dye}) was calculated using the following formula: $M_{dye} = (A_{685conjugate} \times 10)/A_{685dye}$, where $A_{685conjugate}$ was the absorbance of the conjugate at 685 nm and A_{685dye} was the absorbance of 10 μg of BM104 (diluted in the same buffer as the conjugate) at 685 nm.

A competitive ELISA was used to determine the CEA epitope recognized by SGM-ch511. Wells of a microtiter plate were coated with 1 μg/well recombinant CEA (Abcam) in PBS at 22 °C overnight. After washing with PBS and saturation with BSA 10 mg/ml in PBS, a fix quantity of SGM-ch511 (2.5 ng/well) mixed with dilutions (10 μg/ml to 1 ng/ml) of the reference mouse mAbs against the CEA epitopes Gold 1 to 5 (B93, 35A7, B17, CE25, and 192, respectively [22]) or the parental mAb 511 was added to the wells and incubated at 37 °C for 2 h. After washings, SGM-ch511 binding to CEA was revealed using peroxidase-labeled goat anti-human IgG and o-phenylenediamine dihydrochloride (OPD) followed by reading at 490 nm.

Quantum yield (QY) was measured according to Würth C et al. [23] using 5 μM of SGM-101, control conjugates and free dyes. After absorbance and fluorescence measurements, QY was calculated using the formula: $\phi_x = \phi_s\,(F_x/F_s)\ x\ (f_s/f_x)\ x\ (n^2{}_x/n^2{}_s)$, where ϕ_x was the sample QY, ϕ_s was the standard QY (= 0.39 for Alexa Fluor® 680 in PBS), F_x was the integrated intensity of fluorescence of the sample, F_s was the integrated intensity of fluorescence of the standard, f_s the absorption factor of the standard ($=1-10^{-\text{Absorbance value}}$), f_x the absorption factor of the sample ($=1-10^{-\text{Absorbance value}}$), n_x the refractive index of the sample buffer (e.g., n = 1.4305 for DMF, n = 1.334 for PBS) and n_s was the refractive index of the standard buffer (1.334 for PBS).

Photobleaching of the different conjugates (diluted to the same concentration) was measured after exposure to clinical wavelengths (*i.e.* wavelengths of the different devices approved for FGS). After exposure, fluorescence emission was registered with a spectrofluorimeter.

SGM-101 was radiolabeled with 2.5 μCi/μg ^{125}I by using the iodogen method (Pierce, Rockford, IL). Free ^{125}I was separated from labeled SGM-101 through a PD-10 column (GE Healthcare) equilibrated in PBS. The specific activity of the radiolabeled conjugate ranged from 1 to 2 μCi/μg. For binding analysis, 20 ng of ^{125}I-SGM-101 was mixed with CEA or an irrelevant antigen coupled to Sepharose beads and incubated at 37 °C with agitation overnight. The binding ratio was calculated using the following equation: cpm of the bound fraction (after bead washing)/cpm of the total fraction (before bead washing).

Mass spectrometry was performed using a 4800 Maldi TOF/TOF Analyzer (AB Sciex) and the 4000 Series Explorer™ software (AB Sciex). After dialysis against PBS just before analysis, diluted