Radioiodinated anti-DNA-histone 1 complex chimeric tumor necrosis therapy (TNT) monoclonal antibody 1

[$^{125/131}$I]-chTNT-1/B

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Chemical name:	Radioiodinated anti-DNA-histone 1 complex chimeric tumor necrosis therapy (TNT) monoclonal antibody 1	
Abbreviated name:	[$^{125/131}$I]-chTNT-1	
Synonym:	[^{131}I]-chTNT; Cotara®	
Agent Category:	Antibody	
Target:	DNA-histone 1 complex	
Target Category:	Nucleic acids-protein	
Method of detection:	Single-photon emission computed tomography (SPECT); gamma planar imaging	
Source of signal / contrast:	$^{125/131}$I	
Activation:	No	
Studies:	• *In vitro* • Rodents • Humans	Structure not available in PubChem.

Background

[PubMed]

Because neoplastic solid tumors grow rapidly, the lack of sufficient blood supply (and nutrition) leads to cell death and lysis in the lesion and results in the development and accumulation of necrotic areas in the affected areas. As a consequence of the lysis, the

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nuclear antigens (e.g., DNA, histone proteins, etc.) of the lysed cells are exposed in the necrotic tissues. Investigators have used the exposed antigens to develop monoclonal antibodies (mAbs) that have been radiolabeled for evaluation in preclinical and clinical studies to detect and treat malignant tumors (1). This treatment protocol is known as tumor necrosis therapy (TNT), and the mAb used for this treatment targets only the intracellular antigens present in the necrotic section of a tumor. Although the intact cell membrane of a viable cell does not allow the passage of mAbs (because of their large size), the lysed membrane of a necrotic cell provides mAbs with free access to bind the intracellular antigens, such as DNA-histone complexes, heterochromatic DNA, and single-strand DNA of the lysed cells (2). More than two decades ago, ^{125}I-labeled F(ab')$_2$ fragments of a mAb designated TNT-1 were shown to bind the nuclear antigens of necrotic cells in xenograft tumors in nude mice. This observation was confirmed with gamma planar imaging using ^{131}I-labeled TNT-1 F(ab')$_2$ fragments (3). In a subsequent study with nude mice bearing ME-180 cell tumors (of human cervical carcinoma origin), the intact [^{131}I]-TNT-1 mAb was shown to have potential utility in the imaging and therapeutic treatment of the lesions, and it was concluded that treatment success was dependent on the number of necrotic cells present in the tumor (i.e., the higher the number of necrotic cells, the better the treatment results) (4). In another study, the TNT-1 mAb was determined to specifically target the histone H1 protein in nuclear extracts of Raji cells (5). Khawli et al. showed that uptake of the ^{125}I-biotinylated chimeric TNT-1 mAb ([^{125}I]-chTNT-1/B) by LS174T (human colon carcinoma cell line) and Madison 109 (murine adenocarcinoma cell line) cell tumors in mice was superior to that of the parent (non-biotinylated) [^{125}I]-chTNT-1 mAb (2). Biotinylation was reported to improve uptake of the mAb by the tumor because it modified the surface charge, and reduced the circulation time of the molecule. In addition, conjugation of biotin to the mAb decreased the isoelectric point of the molecule and improved its pharmacokinetic properties (2). A ^{111}In-labeled chimeric TNT-3 mAb (targets single-strand DNA), as well as its ^{111}In-labeled Fab' and F(ab')$_2$ fragments, were shown to be suitable for the detection of necrotic solid cancerous tumors in mice (6).

The mechanism of action of the radioiodinated TNT mAb is believed to be as follows: the mAb binds to the intracellular antigens present in the necrotic sections of a tumor, and the radioactive iodine bound to the mAb generates enough energy (as 90% β radiation (192 keV) and 10% γ radiation (364.5, keV)) to kill the surrounding tumor cells with little damage to the normal tissue (1). [^{131}I]-chTNT-1/B mAb has also been evaluated in a phase I clinical trial for the treatment of advanced colon and colorectal cancers (7) and is under clinical evaluation for the treatment of high-grade adult gliomas (1). In 2003, the Chinese State Food and Drug Administration (CSFDA) approved the use of [^{131}I]-chTNT-1/B mAb for the immunotherapy of advanced lung cancer in China (8).

Related Resource Links

Clinical trials with [^{131}I]-chTNT-1/B

Modification of proteins PubMed

Antibody Biotinylation

Synthesis

[PubMed]

TNT-1 mAbs used for the preclinical studies were purified from mouse ascites and radioiodinated ($^{125/131}$I) with the Iodogen method as described by Epstein et al. (3). The specific activity of this preparation was reported to be 37 MBq/6.6 μmol (1 mCi/μmol) (4). The radiochemical yield (RCY) and radiochemical purity (RCP) of [$^{125/131}$I]-TNT-1 mAb were not reported in these publications.

In another preclinical study, the source of chTNT was not reported; however, the RCY of the purified [^{125}I]-chTNT-1/B was reported to be 90%–95%, and the RCP was >99% as determined with instant thin-layer chromatography (2). The specific activity of this labeled chimeric mAb was not reported. To study the biodistribution of [^{125}I]-chTNT-1/B in rodents, the optimal mAb:biotin ratio for the molecule was determined to be 1:3 (2).

For clinical studies, chTNT was expressed in and purified from suspension cultures of NSO cells, a murine myeloma cell line, following good manufacturing practice procedures (1, 7, 8). The mAb was then biotinylated and labeled with ^{131}I using the chloramine-T method as described elsewhere (2). The specific activity of [^{131}I]-chTNT-1/B was reported to be ~370 GBq/6.6 μmol (10 Ci/6.6 μmol). The RCY and RCP of the labeled product were not reported.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The isoelectric points of chTNT-1 and chTNT-1/B were determined to be >9.6 and 7.2–7.8, respectively (2). Using a radioimmunoassay with Raji cells, the affinity constants of the parent mAb and the biotinylated mAb were reported to be 2.5 nM and 2.0 nM, respectively (2).

Animal Studies

Rodents

[PubMed]

The biodistribution of [^{131}I]-TNT-1 was investigated in nude mice (n = 5 animals/group) bearing ME-180 cell tumors as described by Chen et al. (4). A ^{125}I-labeled mAb (Lym-1; binds the surface antigen of B-lymphocytes) that does not react with the intracellular antigens was used as a control. The animals were infused with a mixture of the radioiodinated mAbs (TNT-1/B plus Lym-1; route of administration not mentioned), and the mice were euthanized at various time points varying from 4 h to 8 days after the treatment. At each time point, the major organs were obtained from the animals and

accumulated radioactivity was determined. In the organs and the tumors, similar biodistribution patterns were observed for both of the radioiodinated mAbs 1 week after administration of the labeled biomolecules. Repeat dosing of the mice (at 1 and 2 weeks after the initial dose), however, followed by gamma planar imaging of the animals 1 week after each dose, revealed that the amount of radioactivity from [^{131}I]-TNT-1 mAb in the tumors had increased after each dose (i.e., the amount of label in the tumors at week 3 was greater than that at week 2, and the amount of label in the tumors at week 2 was greater than that at week 1). No such trend was apparent in the tumors of mice treated with the control radioiodinated mAb. From this study, the investigators concluded that the therapeutic efficacy of chTNT mAbs was dependent on the number of lysed cells present in the necrotic portion of a tumor (4). No blocking studies were reported.

In another study, the biodistribution and pharmacokinetics of [^{125}I]-chTNT-1 and [^{125}I]-chTNT-1/B were compared in nude mice (n = 4–5 animals/group) bearing LS174T cell tumors on the left flank (2). The animals were injected with the labeled mAbs through the tail vein and euthanized 1–3 days postinjection (p.i.) to determine the amount of radioactivity present in various organs and the tumors. Data obtained from this study were presented as percent injected dose per gram tissue (% ID/g). With [^{125}I]-chTNT-1, the amounts of radioactivity in the blood, muscle, and tumor were 5.91 ± 0.40% ID/g, 0.37 ± 0.03% ID/g, and 2.78 ± 0.20% ID/g, respectively at 1 day p.i. With [^{125}I]-chTNT-1/B, the accumulation of label in these organs was 2.53 ± 0.10% ID/g, 0.28 ± 0.04% ID/g, and 3.25 ± 0.36% ID/g, respectively, at 1 day p.i. The tumor/muscle (T/M) ratios for the non-biotinylated and the biotinylated mAbs at 1 day after treatment were 3.19 ± 0.15 and 4.27 ± 1.30, respectively (2). A similar trend for the T/M ratios was observed with both tracers at 3 days p.i. No blocking studies were reported. The circulation half-lives of [^{125}I]-chTNT-1 and [^{125}I]-chTNT-1/B were determined to be 30.40 ± 1.80 h and 17.4 ± 2.40 h, respectively (2).

From these studies, the investigators concluded that biotinylation of chTNT-1 improved its uptake by the tumors compared to the non-biotinylated mAb due to rapid clearance of the biotinylated mAb from circulation (2).

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

In a clinical trial, 107 patients were administered two doses of [^{131}I]-chTNT-1/B either intravenously (i.v.; *n* = 62 patients with different cancers) or as an intratumoral injection (*n* = 45 patients with lung cancer) at 2- to 4-week intervals (9). Whole-body scintigraphic images of the individuals obtained at different time points varying from 0.5 h to 25 days after the treatment revealed retention of radioactivity in the vicinity of the tumors for at least 8 days and 25 days in patients who received the labeled mAb through the i.v. or the intratumoral route, respectively (9). During this study, the biodistribution of the label in the tumor and non-tumor areas was measured in 11 patients from each group. The average radiation dose absorbed by the tumor and the non-tumor lung tissue in patients who received the i.v. treatment was 8.45 Gy and 2.35 Gy, respectively, and the tumor/non-tumor (T/NT) ratio in these patients was 3.8. Patients who received the intratumoral treatment had radiation doses of 30.0 Gy and 2.65 Gy in the tumor and the non-tumor lung tissue, respectively, and the T/NT ratio in these patients was 16.1. Of the patients involved in this study (total 107 patients who received treatment either through i.v. or the intratumoral route), 3.7% (4 patients) showed a complete response (CR), 30.8% (33 patients) had a partial response (PR), 55.1% (59 patients) showed no change, and 10.3% (11 patients) showed progression of the disease. On the basis of this clinical trial, the CSFDA approved the use of [^{131}I]-chTNT-1/B for the treatment of lung cancer in China.

In another clinical trial, 43 patients with advanced lung cancer were treated with two doses of [^{131}I]-chTNT-1/B as described elsewhere (10). Among these patients, 22 individuals received the treatment as an i.v. infusion (Group 1), 16 were given an intratumoral injection of the labeled mAb (Group 2), and 5 patients received 75% of their dose as an i.v. infusion and 25% as an intratumoral injection (Group 3). Biodistribution of the label was investigated with scintigraphy in 39 of the 43 patients (4 individuals could not be evaluated), and the T/NT ratios of patients in the various groups were determined. The T/NT ratios of patients in Groups 1 (*n* = 19 individuals), 2 (*n* = 14 individuals), and 3 (*n* = 3 individuals) were 1.36, 14.6, and 9.38, respectively. At 4–6 days after treatment, background radioactivity was visible in the hearts, large blood vessels, and livers of the Group 1 patients, but little (in the liver and heart) or no tracer was visible in any organ of the Group 2 and 3 patients. Whole-body scintigraphy in a patient with non-small cell lung carcinoma at 1 day and 14 days after an intratumoral injection of [^{131}I]-chTNT-1/B showed that the label was localized in and around the tumor during this period (10). In another patient who received the labeled mAb through an i.v. infusion, whole-body scintigraphy showed that the label was detected in the tumor 1 h after administration, but it was also detected in the heart at this time point. At 3 days and 6 days after treatment, the radioactivity in this patient was present mainly in the lung lesion and in a metastatic tumor of the leg. The response rate (CR + PR) of the patients in Groups 2 and 3 was 52.4% compared to 9.1% for the patients in Group 1. From this study, the investigators concluded that the intratumoral treatment route used with [^{131}I]-chTNT-1/B was suitable for the treatment of late-stage lung cancer and probably also for the treatment of other cancerous tumors (10).

In a phase I clinical trial approved by the United States Food and Drug Administration (FDA), 21 patients with advanced colon or colorectal cancer were given an i.v. infusion of

12.95 to 66.23 MBq/kg (from 0.35 to 1.79 mCi/kg) [^{131}I]-chTNT-1/B in cohorts of three individuals (7). Whole-body single-photon emission computed tomography (SPECT) imaging was performed on the patients at various time points ranging from 1 h to 6 days after treatment, and the dose uptake in tumors and the normal organs was calculated from the images for 12 patients as described by Street et al. (7). The radiation doses in these patients were <3.2 cGy/mCi and 0.726 cGy/mCi for the normal organs and the bone marrow, respectively. The tumors received an average dose of 12.6 ± 8.7 cGy/mCi, and the mean tumor dose was 616 cGy/mCi for individuals infused with 1.36 mCi/kg of the labeled mAb (n = 2 patients), 1,631 cGy/mCi for those treated with 1.57 mCi/kg (n = 3 patients), and 2,534 cGy/mCi for patients who received 1.79 mCi/kg (n = 3 individuals). The mean whole-body radiation dose (n = 12 patients) was 0.627 ± 0.270 cGy/mCi, and the blood clearance half-life and the whole-body effective half-life of [^{131}I]-chTNT-1/B were 35.0 ± 9.1 h and 53.3 ± 17.1 h, respectively. Primary tumors were visible only in images obtained from patients who received a dose of >58.09 MBq/kg (1.57 mCi/kg) [^{131}I]-chTNT-1/B, which was attributed to the availability of high amounts of the mAb (14–24 mg total dose) in these individuals (7).

The use of [^{131}I]-chTNT-1/B has also been evaluated for the treatment of 51 patients with different types of malignant glioma in phase I and phase II clinical trials approved by the FDA (1, 11). Individuals enrolled in the study were treated with the radioiodinated mAb using the convection-enhanced delivery procedure that delivered [^{131}I]-chTNT-1/B directly into the resection cavity as described elsewhere (11). In the phase I study, it was shown that a dose of >13,000 cGy could be delivered to the tumor, and the lesion retained 34 ± 9% of the dose at 24 h after treatment with a half-life of 46 ± 6 h. Magnetic resonance imaging and SPECT imaging of two patients showed that the labeled mAb was confined to the clinical targeted area of the glioma tumors in these individuals. In a subset of six malignant glioma patients in the phase II study, the local injection of 13.2–71.1 mCi radioactive [^{131}I]-chTNT-1/B was calculated to produce an absorbed tumor dose of 5,492–13,573 cGy (11). A phase II clinical trial to confirm the dose of [^{131}I]-chTNT-1/B required to treat glioblastoma multiforme at first relapse is currently in progress; no results have been published to date (1).

Supplemental Information

[Disclaimers]

No supplemental information is currently available.

References

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