Toxicity and Efficacy of Radioimmunotherapy in Carcinoembryonic Antigen-producing Medullary Thyroid Cancer Xenograft: Comparison of Iodine 131-labeled F(ab’)2 and Pretargeted Bivalent Hapten and Evaluation of Repeated Injections

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Abstract

This study compared the toxicity and efficacy of 131I-labeled bivalent hapten pretargeted by anti-carcinoembryonic antigen (CEA)/anti-No-(diethylenetriamine-N,N',N''-tetraacetic acid-Indium(F6-734) bispecific antibody (AES) reagents] with 131I-labeled anti-CEA F(ab')2 (131I-F6) in mice grafted with a human medullary thyroid carcinoma. Repeated injections of AES reagents were also evaluated.

Mice bearing TT tumor xenografts were treated with 37, 74, or 92.5 MBq of AES reagents, two injections of 74 MBq of AES reagents 45 days apart, or 37 or 92.5 MBq of 131I-F6. Control groups were treated with nonspecific 131I-labeled F(ab')2, nonspecific AES reagents, nonradiolabeled F6, F6-734 bispecific antibody, and nonradiolabeled bivalent hapten or received no injection. For AES treatments, bispecific antibody was injected 48 h before the hapten. Animal weight, hematological toxicity, tumor volume, and serum thyrocalcitonin were monitored during 5 months.

At 92.5 MBq, weight loss was significantly lower after AES than F6 treatment (P = 0.004). The percentages of leukocyte count changes were significantly lower after AES than F6 at 37 and 92.5 MBq (P = 0.01 and 0.04, respectively). The percentage of platelet count changes was significantly lower with AES at the 92.5-MBq dose level (P = 0.04). In the group injected twice with AES reagents, toxicity was not significantly increased after the second treatment. Tumor response was observed in all cases but was significantly longer with repeated treatments of 74 MBq AES reagents than with a single treatment (P = 0.004). Two complete responses were observed with repeated treatments. Changes in thyrocalcitonin level paralleled those in tumor volume.

These results indicate that pretargeted radioimmunotherapy was at least as efficient as one-step radioimmunotherapy and markedly less toxic. Repeated treatments with AES reagents increased efficacy without increasing toxicity.

Introduction

MAbs directly labeled with iodine 131 have given very promising results in the RIT of low-grade lymphomas but have proved only modestly effective for RIT of solid tumors. Activity uptake is generally <0.01% of injected dose per gram in large tumors, and tumor-to-normal tissue ratios are moderate (usually <10; Ref. 1). The AES, a pretargeting technique using a BsMAb and a bivalent hapten, has increased tumor-to-normal tissue ratios in animal models and clinical studies by lowering radioactivity levels 3-5-fold in normal tissues (2-4). Residual or metastatic forms of MTC are slightly chemo-sensitive and thus constitute a potential application for RIT (5). Moreover, it has been clearly determined that MTC neoplastic cells express and secrete CEA (6). A previous study, in which the biodistribution of an anti-CEA/anti-DTPA-indium BsMAb (F6-734) [prepared by chemical coupling of F(ab')2 fragments of an anti-CEA MAb (F6) and an anti-DTPA-indium MAb (734)] plus 125I-labeled di-DTPA-TL was compared with that of an anti-CEA F6 fragment in a human MTC xenograft model, found that ratios for tumor to blood, liver, and kidney at 24 h were, respectively, 20.5, 4.2, and 2.9 times greater with the two-step than the one-step system for an equivalent level of tumor uptake (7). The present study performed in nude mice grafted s.c. with human MTC cell line TT compared the toxicity and efficacy of therapeutic injections (37 and 92.5 MBq) of F6-734/125I-di-DTPA-TL with those of the F(ab')2 fragment of the same antibody directly labeled with iodine 131. The toxicity of repeated treatments with 74 MBq of F6-734/125I-di-DTPA-TL

1 The abbreviations used are: MAb, monoclonal antibody; RIT, radioimmunotherapy; AES, affinity-enhancement system; BsMAb, bispecific monoclonal antibody; MTC, medullary thyroid carcinoma; CEA, carcinoembryonic antigen; di-DTPA-TL, No-(diethylenetriamine-N,N',N''-tetraacetic acid-N'-acetyl)-tyrosyl-Nε-(diethylenetriamine-N,N',N''-tetraacetic acid-N'-acetyl)-lysine complexed with non-radioactive indium; TCT, thyrocalcitonin; DTPA, diethylenetriamine-N,N',N''-tetraacetic acid.

2 To whom requests for reprints should be addressed, at Institut National de la Santé et de la Recherche Médicale, Research Unit 463, Institut de Biologie, 9 quai Moncoussu, 44093 Nantes Cédex 1, France. Phone: (33) 2-40-08-47-47; Fax: (33) 2-40-35-66-97.
was also evaluated, and the efficacy of this therapeutic protocol was compared with that of a single injection of 74 MBq of F6-734/131I-di-DTPA-TL.

Materials and Methods

Cell Line. The human MTC TT cell line obtained from the American Type Culture Collection (Manassas, VA) expresses CEA at its surface and secretes TCT. It was cultivated in adherent cell monolayers in RPMI 1640 medium (Life Technologies, Cergy-Pontoise, France), to which was added 10% FCS (Life Technologies), 1% glutamine (t-glutamine, 200 mm; Life Technologies) and 1% antibiotic (penicillin, 100 U/ml; streptomycin, 100 U/ml; Life Technologies).

Animal Model. Nude mice >10 weeks of age were grafted s.c. in the right flank with 10⁶ TT cells in 0.3 ml of sterile physiological serum. The animals were housed under aseptic conditions and used ~6 weeks after inoculation when tumors were ~200 mm³. Lugol’s solution (0.1%) was added to drinking water (1 ml/100 ml) the week before and then 2 weeks after injection of the radioidinated reagent.

Antibody and Hapten. The reference antibody was an anti-CEA MAb designated F6. This mouse IgG1 antibody, used in F(ab′)₂ fragment form (8), was kindly provided by CIS Bio International (Gif sur Yvette, France). The F6-734 BsMAb, obtained by chemical coupling of the Fab' fragment of F6 antibody with the Fab' fragment of 734 antibody (anti-DTPA-iodium IgG1), was developed and kindly provided by Immunotech (Marseille, France). The irrelevant BsMAb used as a control was a mixture of a BsMAb obtained by coupling the Fab' fragment of the F6 anti-CEA antibody to the Fab' fragment of the 679 MAb (a murine anti-histamine-succinyl-glycine IgG1) and of a BsMAb prepared with the Fab' fragment of the G7A5 MAb (a murine anti-melanoma IgG1) and the Fab' fragment of the 734 anti-DTPA-iodium antibody. The F(ab′)₂ fragment of the 734 anti-DTPA-iodium antibody served as a control for directly labeled F(ab′)₂. These antibodies were developed and kindly provided by Immunotech, together with the bivalent hapten di-DTPA-TL (2).

Labeling and Controls of the F6 Fragment. Fragments were labeled using iodogen, as described by Farker and Speck (9). The specific activity of 131I-F6, measured in an ionization chamber (Medi-202; Medisyséme, Guyancourt, France) ranged from 185 to 555 MBq/mg for the different groups. The radiochemical purity of 131I-F6, as determined by TLC on instant TLC-SG chromatography paper (Gelman, Ann Arbor, MI), was always >98%. The immunoreactivity of 131I-F6, measured in an ionization chamber (Medi-202; Medisystéme, Guyancourt, France) ranged from 185 to 555 MBq/mg for the different groups. The radiochemical purity was >90%.

Labeling and Controls of di-DTPA-TL. Labeling and controls were performed as described previously (10). In a sterile 2-ml plastic tube were deposited successively 25 μl of di-DTPA-TL-In (25 nmol), 25 μl of 0.3 M phosphate buffer (pH 6), 50 μl of a 1-mg/ml chloramine-T solution in 0.3 M phosphate buffer (pH 6), and 100 μl of Na131I at 16.650 GBq/ml in 0.1 M sodium bicarbonate (pH 8; 131I-S3B; CIS Bio International). After 10 min of incubation at room temperature, the reaction was stopped by addition of 1 mg/ml sodium metabisulfite in 0.3 M phosphate buffer (pH 6). The pH of the solution was brought to between 5 and 6 by addition of 750 μl of 1 M n-(2-hydroxyethyl)-piperazine-N'-2-ethane sulfonic acid. The resulting solution was purified on a C18-grafted silica column (Sepack-C18; Millipore, St. Quentin Yvelines, France). Free iodine was eluted with 5 ml of 0.1 M phosphate buffer (pH 7), and the radiolabeled hapten was eluted with 5 ml of a 3:2 mixture of 0.1 M phosphate buffer (pH 7) and ethanol. The specific activity was 59.2–70.3 MBq/nmol, and the radiochemical purity was >90%.

RIT. A total of 10 groups of five to eight mice each were injected i.v. in the lateral tail vein with 0.2 ml of diluted F(ab′)₂, BsMAb, or hapten in sterile physiological serum as follows. Two groups with initial tumor volumes of 180 ± 88 and 245 ± 123 mm³ were injected, respectively, with 37 and 92.5 MBq (250 μg) of 131I-F6 fragment. Three other groups with initial tumor volumes of 302 ± 50, 144 ± 81, and 170 ± 55 mm³ were injected, respectively, with 2, 3, and 4 nmol of BsMAb F6-734 and, 48 h later, with 37, 74, and 92.5 MBq (1, 1.5, and 2 nmol) of 131I-di-DTPA-TL. One other group with an initial tumor volume of 82 ± 46 mm³ received two successive treatments with AES reagents: 3 nmol of F6-734 at day 0, 74 MBq of 131I-di-DTPA-TL at day 2, 3 nmol of F6-734 at day 45, and 74 MBq of 131I-di-DTPA-TL at day 47. Preliminary studies determined that 4 nmol of BsMAb did not saturate the tumor binding sites and that a molar ratio of 0.5 between the hapten and BsMAb was most favorable for targeting (11). Four control groups were treated, respectively, with (a) 92.5 MBq of non-specific 131I-F6 fragment, (b) 92.5 MBq of 131I-di-DTPA-TL hapten administered 48 h after a mixture of 4 nmol of irrelevant F6-679 BsMAb and 4 nmol of irrelevant G7A5-734 BsMAb, (c) 250 μg of nonradiolabeled F6 fragment, and (d) 2.5 nmol of F6-734 BsMAb and, 48 h later, 1.25 nmol of nonradiolabeled hapten. The initial tumor volumes of these four groups were, respectively, 113 ± 55, 167 ± 50, 228 ± 94, and 192 ± 125 mm³. Finally, a control group of 12 mice with an initial tumor volume of 228 ± 94 mm³ received no injection.

The length (L), width (W), and thickness (T) of tumors were measured twice a week for 160 days. Tumor volume (V) was calculated according to the formula: \( V = \frac{1}{6} \pi L \times W \times T \). Animals were weighed on the day of injection and then twice a week for 160 days. Biological monitoring was performed on blood samples drawn from the inner border of the eye. The parameters used to evaluate the toxicity of each type of treatment were maximal weight loss and the variation in the number of leukocytes and platelets measured on days 0, 15, 30, and 60. The parameters used to evaluate the efficacy of each type of treatment were minimal relative tumor volume, growth delay (the time required for the tumor to double in size after measurement on the day of treatment), tumor response duration, and the variation in serum TCT concentrations measured by RIA every other week for 5 months (calcitonin immunoradiometric assay; CIS Bio International; Ref. 10).

Statistical Analysis. Owing to the limited number of animals, the means of the quantitative variables for the different groups were compared using nonparametric tests (Mann-Whitney U test for comparison of two groups, the Kruskall-Wallis test).
**Table 1** RIT toxicity after injections of 37 and 92.5 MBq

<table>
<thead>
<tr>
<th>Injected activities and reagents</th>
<th>Maximal weight loss (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Leukocyte variation (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Platelet variation (%)&lt;sup&gt;b&lt;/sup&gt;</th>
<th>No. of mice that died</th>
</tr>
</thead>
<tbody>
<tr>
<td>37 MBq 131I-F6</td>
<td>8 (5–14)</td>
<td>−98 ± 03</td>
<td>−50 ± 26</td>
<td>0</td>
</tr>
<tr>
<td>F6-734/131I-di-DTPA-TL</td>
<td>5 (1–10)</td>
<td>+11 ± 80</td>
<td>+45 ± 64</td>
<td>2 D&lt;sup&gt;36&lt;/sup&gt;, D&lt;sup&gt;67&lt;/sup&gt;</td>
</tr>
<tr>
<td>92.5 MBq 131I-F6</td>
<td>16 (5–25)</td>
<td>−89 ± 08</td>
<td>−66 ± 10</td>
<td>2 D&lt;sup&gt;28&lt;/sup&gt;, D&lt;sup&gt;75&lt;/sup&gt;</td>
</tr>
<tr>
<td>F6-734/131I-di-DTPA-TL</td>
<td>5 (0–11)</td>
<td>−34 ± 41</td>
<td>−39 ± 24</td>
<td>2 D&lt;sup&gt;52&lt;/sup&gt;, D&lt;sup&gt;75&lt;/sup&gt;</td>
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</tbody>
</table>

<sup>a</sup> Range in parentheses.
<sup>b</sup> Percent of variation between the nadir at day 15 and the basal value at day 0.

<sup>D</sup>, day of death.

**Table 2** Blood cell counts at different times after injections of 37 or 92.5 MBq and after repeated injections of 74 MBq

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Day 0</th>
<th>Day 15</th>
<th>Day 30</th>
<th>Day 60</th>
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<tbody>
<tr>
<td>Leukocytes (10&lt;sup&gt;6&lt;/sup&gt;/mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>37 MBq 131I-F6</td>
<td>2480 ± 454</td>
<td>50 ± 100</td>
<td>2075 ± 1021</td>
<td>2425 ± 531</td>
</tr>
<tr>
<td>F6-734/131I-di-DTPA-TL</td>
<td>1900 ± 806</td>
<td>1640 ± 838</td>
<td>2840 ± 1290</td>
<td>3220 ± 988</td>
</tr>
<tr>
<td>92.5 MBq 131I-F6</td>
<td>2525 ± 2483</td>
<td>175 ± 50</td>
<td>1550 ± 759</td>
<td>1700 ± 816</td>
</tr>
<tr>
<td>F6-734/131I-di-DTPA-TL</td>
<td>2200 ± 535</td>
<td>1560 ± 1160</td>
<td>1580 ± 1100</td>
<td>2450 ± 900</td>
</tr>
<tr>
<td>74 MBq F6-734/131I-di-DTPA-TL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First treatment</td>
<td>3871 ± 2018</td>
<td>943 ± 605</td>
<td>4314 ± 1704</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Second treatment</td>
<td>3642 ± 1446</td>
<td>1542 ± 672</td>
<td>3428 ± 2283</td>
<td>5571 ± 7145</td>
</tr>
<tr>
<td>Platelets (10&lt;sup&gt;6&lt;/sup&gt;/mm&lt;sup&gt;3&lt;/sup&gt;)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>37 MBq 131I-F6</td>
<td>1.70 ± 0.55</td>
<td>0.71 ± 0.18</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.01 ± 0.26</td>
</tr>
<tr>
<td>F6-734/131I-di-DTPA-TL</td>
<td>1.18 ± 0.53</td>
<td>1.46 ± 0.52</td>
<td>2.42 ± 0.89</td>
<td>1.50 ± 0.20</td>
</tr>
<tr>
<td>92.5 MBq 131I-F6</td>
<td>1.47 ± 0.25</td>
<td>0.48 ± 0.11</td>
<td>1.23 ± 0.65</td>
<td>1.28 ± 0.65</td>
</tr>
<tr>
<td>F6-734/131I-di-DTPA-TL</td>
<td>1.12 ± 0.58</td>
<td>0.73 ± 0.38</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.80 ± 0.50</td>
</tr>
<tr>
<td>74 MBq F6-734/131I-di-DTPA-TL</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>First treatment</td>
<td>1.63 ± 0.81</td>
<td>1.14 ± 0.64</td>
<td>0.92 ± 0.26</td>
<td>ND&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Second treatment</td>
<td>0.88 ± 0.14</td>
<td>0.92 ± 0.36</td>
<td>0.84 ± 0.28</td>
<td>0.94 ± 0.39</td>
</tr>
</tbody>
</table>

<sup>a</sup> ND, not done.

Blood cell counts are indicated in Table 2. In untreated controls, the mean leukocyte count was 2700/mm<sup>3</sup> (range, 800-7000) and that of platelets was 1.4 × 10<sup>6</sup>/mm<sup>3</sup> (range, 0.57–2.7 × 10<sup>6</sup>). The percentage of leukocyte count changes (between the nadir at day 15 and the basal value at day 0) was significantly different between groups treated with 131I-F6 and that treated with F6-734/131I-di-DTPA-TL at 37 MBq (P = 0.01) and at 92.5 MBq (P = 0.04; Table 1). For platelet toxicity, no significant difference was found between the group treated with 37 MBq of 131I-F6 and that treated with 37 MBq of F6-734/131I-di-DTPA-TL (P = 0.08). Conversely, the difference between the group treated with 92.5 MBq of 131I-F6 and that treated with 92.5 MBq of F6-734/131I-di-DTPA-TL was significant (P = 0.04; Table 1).

Six animals died during the monitoring period (Table 1). Deaths occurring 28 days after injection of 92.5 MBq of 131I-F6 were related to leukopenia. The others occurred later and were related to infection (as confirmed by histological study at autopsy).

**Results**

**Toxicity in Groups Treated with 37 or 92.5 MBq of 131I-F6 or F6-734/131I-di-DTPA-TL.** In untreated controls, mean weight loss was 3% (range, 2–7%). No significant difference in weight loss was found between the group treated with 37 MBq of 131I-F6 and that treated with 37 MBq of F6-734/131I-di-DTPA-TL (P = 0.12; Table 1). Conversely, the difference between the group treated with 92.5 MBq of 131I-F6 and that treated with 92.5 MBq of F6-734/131I-di-DTPA-TL was significant (P = 0.004).

Histological analyses of untreated tumors and of tumors treated with AES reagents were performed. The fragments were fixed in 10% formal solution, embedded in paraffin, cut into 4-μm sections, and stained with hemalun-eosin-safran. Cellular reactivity with anti-CEA MAb was studied by an indirect immunoperoxidase technique.

**Toxicity in the Group Treated Twice (45 Days Apart) with 74 MBq of F6-734/131I-di-DTPA-TL.** The toxicity of the second treatment was not significantly greater than that of

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*test for comparison of more than two groups, and the Wilcoxon matched pairs signed rank sum test for comparison of two treatments in the same animals). P = 0.05 was considered significant. BMDP (Cork, Ireland) statistical software, version 7.0, was used for the analysis.

**Histological Study.** Histological analyses of untreated tumors and of tumors treated with AES reagents were performed. The fragments were fixed in 10% formal solution, embedded in paraffin, cut into 4-μm sections, and stained with hemalun-eosin-safran. Cellular reactivity with anti-CEA MAb was studied by an indirect immunoperoxidase technique.

**Results**

Toxicity in groups treated with 37 or 92.5 MBq of **131I-F6 or F6-734/131I-di-DTPA-TL.** In untreated controls, mean weight loss was 3% (range, 2–7%). No significant difference in weight loss was found between the group treated with 37 MBq of **131I-F6** and that treated with 37 MBq of F6-734/131I-di-DTPA-TL (P = 0.12; Table 1). Conversely, the difference between the group treated with 92.5 MBq of **131I-F6** and that treated with 92.5 MBq of F6-734/131I-di-DTPA-TL was significant (P = 0.004).

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Six animals died during the monitoring period (Table 1). Deaths occurring 28 days after injection of 92.5 MBq of **131I-F6** were related to leukopenia. The others occurred later and were related to infection (as confirmed by histological study at autopsy).

**Toxicity in the Group Treated Twice (45 Days Apart) with 74 MBq of F6-734/131I-di-DTPA-TL.** The toxicity of the second treatment was not significantly greater than that of
Efficacy of RIT. Tumor responses were observed in all animals treated with $^{131}$I-F6 and F6-734/$^{131}$I-di-DTPA-TL (Figs. 1 and 2). No complete responses were obtained in the groups treated with a single dose of RIT, whereas two of seven treated animals had complete long-lasting (154 days) responses in the group treated twice with 74 MBq of F6-734/$^{131}$I-di-DTPA-TL 45 days apart. Minimal relative tumor volume, tumor growth delay, and response duration for each group are shown in Table 3. Tumor growth delays in these treated groups were significantly longer than in the untreated group. The changes in TCT concentrations (Figs. 3 and 4) paralleled those in tumor volumes.

A comparison of tumor responses in groups treated with 37 or 92.5 MBq of $^{131}$I-F6 and F6-734/$^{131}$I-di-DTPA-TL (37 MBq of $^{131}$I-F6 serving as reference) showed that the only significant variable was longer growth delay after injection of 92.5 MBq of F6-734/$^{131}$I-di-DTPA-TL (Table 3). The mean initial tumor volumes were not significantly different between this group and the reference ($P = 0.41$). A comparison of tumor responses in groups treated once or twice with 74 MBq of F6-734/$^{131}$I-di-DTPA-TL showed that minimal relative tumor volume was not significantly lower in the group treated twice ($P = 0.09$), but that response duration and tumor growth delay (for partial responses alone) were significantly longer ($P = 0.004$ and 0.01, respectively). The mean initial tumor volumes were not significantly different between these two groups ($P = 0.17$).

In the control groups, rapid tumor growth was associated with a rise in serum TCT concentration (Figs. 1 and 3). Growth delays were not significantly different between these groups ($P > 0.05$; Table 3).

Histological Study. Tumor proliferation was microscopically comparable in the different samples obtained from growing tumors, consisting of a dense growth of large cells with a high nucleocytoplasmic ratio. Untreated tumor was characterized by an absence of necrosis, and anti-CEA antibody labeled 100% of the cells. Recurring tumors after one or two RITs...
with therapeutic activities of AES reagents (pretargeting interval present study, hematological toxicity was significantly lower if a shorter interval is used. In the latter case, tumor localization is maximal, but the tumor-to-blood ratio is reduced (3, 4). In the

Discussion

Studies in RIT have clearly shown that directly labeled MAbs generally induce elevated hematotoxicity because of the persistence of high levels of radioactivity in the bloodstream (3, 11). Hematotoxicity can be reduced by targeting small molecules to antibodies prelocalized on the surface of tumor cells, thereby improving uptake selectivity and reducing radioactivity in normal tissues (2-4, 12). With AES reagents, the selectivity of tumor targeting may be improved by using a long (48-h) interval between injections of BsMAb and hapten. Alternatively, very high irradiation doses may be delivered to the tumor if a shorter interval is used. In the latter case, tumor localization is maximal, but the tumor-to-blood ratio is reduced (3, 4). In the present study, hematological toxicity was significantly lower with therapeutic activities of AES reagents (pretargeting interval of 48 h) than with an equal activity of directly labeled F(ab')2, and a second administration of AES reagents did not cause increased toxicity. The efficiency of internal radiotherapy often depends on repeated administration of radiopharmaceuticals, particularly in the treatment of neuroendocrine tumors (13), and the management of cancers at a metastatic stage generally requires the association of several potentially synergistic treatments. The AES appears to be particularly suitable for repeated therapy and combination therapy with chemotherapeutic agents that can also induce myelosuppression.

Several groups have reported partial or complete tumor remissions after RIT in animal models of xenografted solid tumors such as colorectal carcinomas (3, 14). In the present study, significantly longer growth delays than those for the untreated group were obtained with $^{131}$I-F6 and F6-734/$^{131}$I-di-DTPA-TL. However, no complete responses were observed after a single treatment, and tumors relapsed with the same endocrine differentiation in all cases. As shown for other antigens such as TAG-72 and MUC-1 in adenocarcinoma cell lines (11, 14), 80-100% of the TT cells relapsing after RIT still expressed CEA. Thus, the failure of RIT to cure the animals entirely was not attributable to an outgrowth of antigen-negative cell subpopulations, so that repeated treatments with RIT were possible. The second treatment was performed when approximately half of the tumors had resumed growth after treatment with a single injection of 74 MBq of F6-734/$^{131}$I-di-DTPA-TL. This resulted in a significant increase in the duration of tumor response, and two animals with initial tumors <50 mm$^3$ showed a complete response during 22 weeks. It would appear that the efficacy of the second injection was greater than that of the first. Tumor response in the group treated twice was significantly greater than a doubling of the duration observed after a single injection of $^{131}$I-F6 and F6-734/$^{131}$I-di-DTPA-TL. However, no complete responses were observed after a single treatment, and tumors relapsed with the same endocrine differentiation in all cases. As shown for other antigens such as TAG-72 and MUC-1 in adenocarcinoma cell lines (11, 14), 80-100% of the TT cells relapsing after RIT still expressed CEA. Thus, the failure of RIT to cure the animals entirely was not attributable to an outgrowth of antigen-negative cell subpopulations, so that repeated treatments with RIT were possible. The second treatment was performed when approximately half of the tumors had resumed growth after treatment with a single injection of 74 MBq of F6-734/$^{131}$I-di-DTPA-TL. This resulted in a significant increase in the duration of tumor response, and two animals with initial tumors <50 mm$^3$ showed a complete response during 22 weeks. It would appear that the efficacy of the second injection was greater than that of the first. Tumor response in the group treated twice was significantly longer than a doubling of the duration observed after a single injection. ($P = 0.004$). Thirty to 75% of the surface of the tumor showed necrosis and fibrosis at the time of the second treatment. Five of seven mice had a smaller tumor mass at the time of the second treatment than initially. This is apparently conducive to higher antibody uptake, which provides probably a higher delivered dose (15, 16). The two tumors that were larger at the time of the second treatment probably had a higher percentage of cycling cells and thus greater radiation sensitivity (10).

No cures were achieved, although tumor response im-

### Table 3 Tumor effect in groups of mice treated with single or repeated doses of RIT and in control groups

<table>
<thead>
<tr>
<th>Groups of mice</th>
<th>Minimal relative tumor volume (%)</th>
<th>Growth delay (days)</th>
<th>Response duration (days)</th>
<th>$P^{ab}$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$^{131}$I-F6 (37 MBq)</td>
<td>63 ± 14</td>
<td>44 ± 21</td>
<td>17 ± 04</td>
<td></td>
</tr>
<tr>
<td>F6-734/$^{131}$I-di-DTPA-TL (37 MBq)</td>
<td>65 ± 28</td>
<td>57 ± 09</td>
<td>31 ± 06</td>
<td>0.60</td>
</tr>
<tr>
<td>$^{131}$I-F6 (92.5 MBq)</td>
<td>42 ± 27</td>
<td>65 ± 11</td>
<td>30 ± 07</td>
<td>0.18</td>
</tr>
<tr>
<td>F6-734/$^{131}$I-di-DTPA-TL (92.5 MBq)</td>
<td>42 ± 18</td>
<td>86 ± 22</td>
<td>39 ± 08</td>
<td>0.03</td>
</tr>
<tr>
<td>F6-734/$^{131}$I-di-DTPA-TL (74 MBq)</td>
<td>61 ± 23</td>
<td>56 ± 09</td>
<td>30 ± 08</td>
<td></td>
</tr>
<tr>
<td>F6-734/$^{131}$I-di-DTPA-TL (74 MBq × 2)</td>
<td>29 ± 32</td>
<td>121 ± 12'</td>
<td>111 ± 31</td>
<td></td>
</tr>
<tr>
<td>No injection</td>
<td>100 ± 0</td>
<td>12 ± 04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonradiolabeled F6</td>
<td>100 ± 0</td>
<td>9 ± 02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nonradiolabeled F6-734/di-DTPA-TL</td>
<td>100 ± 0</td>
<td>23 ± 10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>$^{131}$I-734</td>
<td>100 ± 0</td>
<td>19 ± 04</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Irrelevant BsMab/$^{131}$I-di-DTPA-TL</td>
<td>100 ± 0</td>
<td>12 ± 04</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

$^a$ Ratio of the smallest tumor volume observed to the initial size before treatment.

$^b$ $P$, comparison of growth delay with reference treatment ($^{131}$I-F6 37 MBq).

$^c$ Calculated for mice showing partial responses only.

$^d$ No tumor shrinkage occurred in these mice.
proven after the second administration of AES reagents. In solid tumors of this size, the distribution of immunoconjugates is heterogeneous. Hypoxic areas, which are the most radioresistant, probably receive insufficient doses for curative purposes (17). Thus, RIT should be combined with other therapeutic modalities. The use of the hypoxia-selective cytotoxin tirapazamine in association with fractionated external irradiation or internal low-dose rate irradiation has provided encouraging results in preclinical models (17). Moreover, in this TT xenograft model, a synergistic antitumor effect was produced by a combination of 131I-labeled anti-CEA IgG with doxorubicin (18). At the maximal tolerated dose of each agent, an 85% cure rate was obtained with bone marrow support. In this study, we have shown that pretargeted RIT with AES reagents was at least as efficient as one-step RIT and markedly less toxic, and that repeated treatments with AES reagents increased therapeutic efficacy without increasing toxicity. It is likely that a combination of AES RIT with cytotoxic and radiosensitizing drugs such as doxorubicin would achieve high response rates in the treatment of MTC without bone marrow support.

Acknowledgments

We thank Marie de Cussé, James Gray, and Alain Maisonneuve for technical assistance.

References


Fig. 3 Variation of TCT concentration (picograms per milliliter) in groups of mice treated with a single dose and in control groups (two mice per group). A, 131I-F6, 37 MBq (○) and 92.5 MBq (●); B, F6-734/131I-di-DTPA-TL, 37 MBq (○) and 92.5 MBq (●); C, no treatment (□), nonradiolabeled F6 (○), and nonradiolabeled F6-734/ di-DTPA-TL (●); D, 131I-734 (○) and irrelevant BsMAB/131I-di-DTPA-TL (●).

Fig. 4 Variation of TCT concentration in groups of mice treated once (- - -) or twice (--) with 74 MBq of F6-734/131I-di-DTPA-TL. Arrows represent times of BsMAB F6-734 administration.
Toxicity and Efficacy of Radioimmunotherapy in Carcinoembryonic Antigen-producing Medullary Thyroid Cancer Xenograft: Comparison of Iodine 131-labeled F(ab’)2 and Pretargeted Bivalent Hapten and Evaluation of Repeated Injections

Françoise Kraeber-Bodéré, Alain Faivre-Chauvet, Catherine Sai-Maurel, et al.

Clin Cancer Res 1999;5:3183s-3189s.

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