Innovations That Influence the Pharmacology of Monoclonal Antibody Guided Tumor Targeting

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Abstract

Tumor targeting by monoclonal antibodies (MAbs) can be enhanced by (a) increasing the percentage of injected dose taken up by the tumor and/or (b) increasing the tumor/nontumor ratios. Several groups have demonstrated that one can increase tumor to nontumor ratios by the use of antibody fragments or the administration of second antibodies. Several other modalities are also possible: (a) the use of recombinant interferon to up-regulate the expression of specific tumor associated antigens such as carcinoembryonic antigen or TAG-72 on the surface of carcinoma cells and thus increase MAb tumor binding has proved successful in both in vitro and in vivo studies; (b) the intracavitary administration of MAbs. Recent studies have demonstrated that when radiolabeled B72.3 is administered i.p. to patients with carcinoma of the peritoneal cavity, it localizes tumor masses with greater efficiency than does concurrent i.v. administered antibody. Studies involving the comparative pharmacology of intracavitary administration of radiolabeled MAb in patients and several animal models will be discussed; (c) it has been reported that prior exposure of hepatoma to external beam radiation will increase radiolabeled MAb tumor targeting. We and others have not been able to duplicate this phenomenon with a human colon cancer xenograft model and radiolabeled MAbs to two different colon carcinoma associated antigens. The possible reasons for these differences will be discussed; (d) the cloning and expression of recombinant MAbs with human constant regions and subsequent size modification constructs will also undoubtedly alter the pharmacology of MAb tumor binding in both diagnostic and therapeutic applications.

Introduction

There are numerous parameters that can have an effect on the pharmacology of MAb4 guided tumor targeting. Some of the more obvious parameters are listed in Table 1. Some of these factors are intrinsic, such as the size and location of the tumor mass, while others can be manipulated. Most of these manipulations involve either (a) increasing the percentage of injected dose of MAb taken up by the tumor or (b) increasing the tumor to nontumor ratios. One of the more widely used procedures of MAb guided tumor targeting involves the use of immunoglobulin Fab and F(ab′)2 fragments (1–3). In general, the use of fragments leads to higher tumor/blood ratios but a lower percentage of injected dose delivered to tumor masses. Similar effects have also been seen with the use of second antibody to clear the blood pool of primary antibody (4); this approach, however, has also led to enhanced liver and spleen uptake.

This paper will outline and review four of many new and innovative approaches to enhance MAb guided tumor targeting. These include: (a) the use of recombinant interferons to up-regulate the expression of specific tumor associated antigens such as carcinoembryonic antigen (CEA) or TAG-72 on the surface of carcinoma cells and thus increase MAb tumor binding; (b) the intracavitary administration of MAbs. Recent studies have demonstrated that when radiolabeled MAb B72.3 is administered i.p. to patients with carcinoma of the peritoneal cavity, it localizes tumor masses with greater efficiency than does concurrent i.v. administered antibody. Studies involving the comparative pharmacology of intracavitary administration of radiolabeled MAb in patients and several animal models will be discussed; (c) it has been reported that prior exposure of hepatoma to external beam radiation will increase radiolabeled MAb tumor targeting. We and others have not been able to duplicate this phenomenon with a human colon cancer xenograft model and radiolabeled MAbs to two different colon carcinoma associated antigens. The possible reasons for these differences will be discussed; (d) the cloning and expression of recombinant MAbs with human constant regions and subsequent size modification constructs will undoubtedly alter the pharmacology of MAb tumor binding.

Augmentation of Tumor Antigen Expression by Recombinant Human Interferons: Enhanced Tumor Targeting of Monoclonal Antibodies

Unlike the antigens that constitute the major histocompatibility complex and receptor molecules, many carcinoma associated tumor antigens are, for the most part, integral components of the cell membrane and do not undergo internalization, capping, etc., after antibody binding. In some cases, the carcinoma associated tumor antigens are expressed by a variety of different tumor types. One example is the B72.3 MAb that was generated in our laboratory using an enriched membrane extract prepared from a human carcinoma metastasis to the liver (5, 6). B72.3 reacts with a high molecular weight glycoprotein antigen, termed TAG-72 (7). The range of reactivity of the B72.3 antibody for carcinoma versus normal is selective; i.e., the only normal adult tissue that shows appreciable reactivity with the antibody is secretory endometrium (8). Other tissues demonstrate minor reactivities (9). In addition, the range of reactivity of B72.3 among different carcinomas is that of a pancarcinoma antigen and includes breast, colorectal, ovarian, stomach, pancreatic, and endometrial cancers (9).

A third characteristic that seems to be shared by many human carcinoma associated antigens is the existence of antigenic heterogeneity among the different tumor lesions. This heterogeneity of tumor antigen expression has been documented both in experimental models using established human carcinoma cell lines and in biopsies prepared from in situ carcinoma lesions (10, 11). Data are available that demonstrate that, within primary and metastatic human carcinomas, not all of the cells may express a given tumor antigen. Although tumor cell heterogeneity can be defined according to a large number of cellular criteria (i.e., cell size, metastatic ability), the heterogeneity of
antigen expression and its potential impact on MAb use for tumor detection and/or therapy will be discussed here.

rHu-IFNs are potent immunomodulatory compounds capable of altering the antigen phenotype of a variety of human cells (12–16). Studies have shown that type I and II rHu-IFNs can amplify the level of expression of class I and II histocompatibility antigens, as well as some specific tumor antigens found on the surface of human melanoma and adenocarcinoma cell populations (see Table 2 and Refs. 15 and 17–27). Previous studies have also demonstrated that different species of recombinant leukocyte interferon (rHu-IFN-αA) can exert different activities, etc.) (28, 29). More recently, we reported that the IFN-β administered to the tumor cell population that express the antigen. Thus, antigenic heterogeneity of tumor masses can perhaps be dealt with using one or a combination of (a) mixtures of MAbs, (b) radionuclides that can kill several cell diameters, and (c) the use of recombinant interferons.

Intracavitary Administration of MAbs

Intracavitary disease such as pleural effusions, ascites, peritoneal implants, or intrathecal tumors are fairly common manifestations of carcinomas. For example, the peritoneal cavity is the primary disease site of ovarian carcinoma, and metastases originating from the breast and colon. Administration of diagnostic tumor targeting agents and therapeutic agents via an intracavitary route provides the theoretical advantages of (a) increasing the concentration of the agent present at the tumor site and (b) decreased toxicity to normal organs such as bone marrow, as well as to those organs that may be involved in immunoglobulin catabolism.

Two studies have recently compared i.p. and i.v. MAb administration in animal models for the detection of peritoneal carcinoma. Using MAb HMFG2 and xenografts of the LoVo human colon carcinoma cell line, Rowlinson et al. (33) have observed that i.p. MAb inoculation is better than i.v. for targeting of peritoneal tumors. Ward et al. (34), using athymic mice bearing both s.c. and i.p. human ovarian carcinoma xenografts, reported enhanced localization to ascites tumor cells after i.p. MAb inoculation but, in contrast to the previous study, observed no advantage of i.p. MAb administration in detection of i.p. solid tumor. In one study, therapy of peritoneal carcinomatosis of human colon cancer xenografts with i.p. 90Y-anticoaginoembryonic antigen MAb has demonstrated a significant decrease in tumor burden 12 days after MAb treatment (35). Hnatowich et al. (36), using i.p. administered 90Y-labeled MAb OC-125 for detection of carcinoma in patients with documented or suspected ovarian cancer, demonstrated tumor:tissue ratios of 3:1 to 25:1. The amount of radioactivity retained in the peritoneum varied among the patients, suggesting individual differences in peritoneal diffusion capabilities which must be taken into account when designing therapeutic protocols. Immunotherapy i.p. using 131I-labeled MAb HMFG2 has been reported in one patient with stage III ovarian carcinoma resistant to chemotherapy (37).

Our laboratory has undertaken studies to: (a) determine the feasibility of i.p. administration of radiolabeled B72.3 for tumor targeting (via both gamma scanning and direct analyses of biopsy specimens); (b) determine the specificity of tumor tar-
Table 2 Summary: interferon enhancement of tumor antigen expression

<table>
<thead>
<tr>
<th>Cell type/antigen(s)</th>
<th>Interferon</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colon: CEA*</td>
<td>Lymphoblastoid</td>
</tr>
<tr>
<td>Melanoma associated antigen</td>
<td>α (Cantell)</td>
</tr>
<tr>
<td>Breast and colon: TAG-72, CEA, M, 90,000</td>
<td>α (clone A)</td>
</tr>
<tr>
<td>Melanoma, M, 80,000</td>
<td>rec. γ</td>
</tr>
<tr>
<td>Ganglioside G02</td>
<td>rec. γ</td>
</tr>
<tr>
<td>Melanoma Cytoplasmic MAA</td>
<td>rec. γ</td>
</tr>
<tr>
<td>Melanoma, M, 32,000, 38,000, 46,000, and 50,000</td>
<td>rec. γ</td>
</tr>
<tr>
<td>Colon, M, 90,000, in vivo localization</td>
<td>rec. α (clone A)</td>
</tr>
<tr>
<td>Neuroblastoma, 4-6 different antigens</td>
<td>rec. γ</td>
</tr>
<tr>
<td>Colon, breast, lung, melanoma, and EBV-transformed B-cells; M111 antigen</td>
<td>rec. γ</td>
</tr>
<tr>
<td>Breast: M, 90,000</td>
<td>rec. α-2b</td>
</tr>
<tr>
<td>Melanoma: 96.5 Ag in vivo patient data</td>
<td>α (Cantell)</td>
</tr>
<tr>
<td>Human serumous effusions: TAG-72, CEA</td>
<td>rec. α (clone A) β (ser) and γ</td>
</tr>
</tbody>
</table>

* CEA, carcinoembryonic antigen; MAA, melanoma associated antigen; EBV, Epstein-Barr virus; rec., recombinant.

Fig. 1. Fluorescence-activated cell sorter analysis of MAb B6.2 binding to the surface of human colon carcinoma cells. Effect of rHu-IFN-αA. The flow cytometric analysis was done, using an Ortho Cytofluorograf System 50H, with blue laser excitation of 200 mW at 488 nm. WiDr cells were incubated for 24–36 h in growth medium with or without 850 antiviral units rHu-IFN-αA/ml, harvested, and incubated in culture medium containing 2 μg MAb B6.2/10⁶ cells for 30 min at 4°C. The cells were then washed thoroughly with Ca²⁺-Mg²⁺-free Dulbecco’s phosphate buffered saline and then treated with fluoresceinated goat anti-mouse IgG (heavy and light chains) (1:30 dilution) for 30 min at 4°C. The cells were washed, excess antibody was removed, and the cells were resuspended at a concentration of 10⁶ cells/ml and stained with propidium iodide (18 μg/ml) and RNase A (2000 units/ml) for 4 h at room temperature. The stained cells were analyzed; under these conditions, propidium iodide bound to nuclear DNA fluoresces red while surface immunofluorescence bound to the M, 90,000 tumor antigen fluoresces green. Data from 25,000 cells were stored on an Ortho Model 2150 computer system and used to generate each 3-dimensional isometric display of DNA content (Y axis), fluorescence intensity, i.e., cell surface expression of the B6.2 reactive M, 90,000 tumor antigen (X axis), and the number of cells (Z axis). A, WiDr cells stained for DNA content but no MAb B6.2; B, WiDr cells stained for nuclear DNA and B6.2 binding; C, WiDr cells treated with 850 antiviral units rHu-IFN-αA for 24–36 h and stained as in B.
regions of the peritoneum. In all such cases, these lesions were identified at surgery as tumor and later confirmed as carcinoma via histopathological examination.

Three of the 10 patients studied were positive for MAb localization via gamma scanning (areas confirmed as tumor at surgery) but were negative for tumor via computer-assisted tomographic scanning and X-ray studies. Lesions as small as approximately 1.5 cm in diameter were clearly defined via gamma scans.

All specimens removed at surgery were analyzed for cpm/kg of tissue. Specimens were then fixed, embedded, and analyzed for percentage of carcinoma cells present. Table 3 shows the RI values (based on % ID MAb uptake per kg of tissue) of the biopsy specimens of tumor and normal tissues from the 10 patients receiving i.p. administered MAB B72.3. An RI of ≥3 was arbitrarily chosen as a "positive" radiolabeled uptake. Eighty-three of 112 (74%) of carcinoma lesions showed RI values ≥3. In some patients with a large tumor burden, as much as 40% of the injected dose was found bound to carcinoma. Note that of the 29 lesions negative for MAb uptake, 11 were from patient MG, the only patient in whom all tumor lesions were negative for TAG-72 antigen expression. Of the 95 histologically confirmed normal tissues biopsied, all but one demonstrated RI values <3 (Table 3). The one biopsy not in this category had an RI of 3.5 and was a histologically normal spleen; this patient also had the highest level of circulating antigen (125I-B72.3 IgG immune complex as determined by high performance liquid chromatography and radioimmunoassay. The other 94 normal tissue biopsies with RI values <3 were from numerous sites, including colon, spleen, ileum, pancreas, lymph node, fallopian tube, liver, ovary, duodenum, and kidney; the vast majority of RI values for normal tissues ranged from 0.5 to 1.5.

Specificity of MAB B72.3 Radiolocalization. To determine if the radiolocalization observed with the i.p. administered 125I-B72.3 IgG was specific, four patients received concomitant infusions (administered i.p.) of 125I-B72.3 IgG and 131I-labeled control MAB BL-3 IgG. B72.3 and BL-3 were administered at the same mg dose and time for each patient. There were some important observations obtained in these analyses concerning "nonspecific" MAb uptake. MAB BL-3 uptake was negative (i.e., RI <3) in 28 carcinoma lesions but showed RI values >3 in 11 others. When the ratios of %ID/kg of these lesions for B72.3 versus BL-3 were analyzed, in none of the 39 lesions was BL-3 uptake greater than that of B72.3. In 8 lesions the ratios were comparable, and in 31 of 39 lesions the B72.3/BL-3 ratios were >2:1, with ratios of >10:1 in the majority of cases; ratios of >100:1, moreover, were observed for many lesions. Thus, while these results demonstrate the specificity of the B72.3 binding, they do point out that "nonspecific" binding of irrelevant MAB to carcinoma lesions may and does indeed occur at times.

Simultaneous i.p. and i.v. Administration of MAB B72.3. Studies were next conducted to determine the relative efficacies of i.p. versus i.v. administered MAB to localize tumor lesions (40). To achieve this goal, patients were concomitantly given 125I-labeled B72.3 i.p. and 131I-labeled B72.3 i.v. Both the i.p. and i.v. administered MAB preparations in each of four patients were identical as far as mg dose and radiolabeling conditions. In 35 or 55 carcinoma lesion biopsies obtained, the i.p. administered B72.3 localized at least 2 times better in terms of %ID/kg than the i.v. administered MAB. In 7 lesions MAB localization was comparable via either route, and in 13 lesions the i.v. administered MAB B72.3 localized at least 2 times better than the i.p. administered MAB. For example, as seen for patient NJ (Table 4), %ID/kg taken up by carcinoma lesions ranged from 11.4 to 45.3 for i.p. administered B72.3 (131I) versus values of only 2.8 to 11.3 %ID/kg for the i.v. administered B72.3 (125I). The ratios of uptake of the i.p. administered MAB were from 2.8 to 7.6 times greater for individual lesions than the i.v. administered MAB. The levels of uptake in the normal tissues of the i.v. and i.p. administered B72.3 IgG were similar (40).

In an attempt to define the reason(s) for the divergence in uptake of i.p. and i.v. administered MAB among different carcinoma lesions, three pathologists independently examined these lesions and characterized them as to various properties. The most striking correlation with differential MAB uptake was whether a given metastasis was a peritoneal implant or "non-implant." Metastatic lesions were characterized as (a) peritoneal implants or (b) nonimplant metastases, i.e., hematogenously borne metastases, lymph node metastases, or local recurrences. For those lesions where all three pathologists did not arrive at the identical conclusion, the lesion was listed as "unclassified." In all 10 nonimplant metastases from three patients, the ratios (%ID/kg) of uptake of the i.v. injected B72.3 were at least 2 times that of the i.p. administered MAB, with ratios ranging up to 38:1. Conversely, in 35 of 40 lesions classified as peritoneal implants, i.p. administered B72.3 localized at least 2 times better than i.v. administered MAB, with the ratios obtained for the majority of lesions being greater than 5:1. Five of 40 peritoneal implants bound comparable levels of i.p. and i.v. administered B72.3.

Pharmacokinetics of Plasma Levels after i.p. MAB Administration. Plasma samples from eight patients studied were obtained before MAB administration and at various time points post-MAB administration (40). The plasma clearance of the i.v. administered 131I-B72.3 IgG and the 125I-B72.3-control IgG were similar, with approximately 50% of the injected dose in the plasma at day 2 and approximately 10% of the injected dose in plasma at day 7 post-MAB administration. In contrast, no more than 30% of the injected dose of i.p. administered B72.3 or BL-3 IgG appeared in the plasma at any point in time, with peak values being obtained at days 2 to 3.

The appearance of 131I-B72.3 IgG in the plasma was noted to be reduced in four of eight patients studied in that 131I-labeled MAB levels in plasma did not rise above 10–12% of injected dose. Pre-MAB administration plasma samples were tested for the B72.3 reactive antigen TAG-72 via RIA as described previously (41, 42) and revealed that sera from these four patients showed the highest levels of circulating TAG-72 antigen.

Several diagnostic and potentially therapeutic applications can be envisioned from these studies. Radiolabeled MABs such

<table>
<thead>
<tr>
<th>Name</th>
<th>RI &lt;3</th>
<th>RI 3-10</th>
<th>RI &gt;10</th>
<th>RI &lt;3</th>
<th>RI 3-10</th>
<th>RI &gt;10</th>
</tr>
</thead>
<tbody>
<tr>
<td>NJ</td>
<td>0</td>
<td>1</td>
<td>17</td>
<td>12</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MH</td>
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<td>4</td>
<td>0</td>
<td>10</td>
<td>0</td>
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</tr>
<tr>
<td>RK</td>
<td>5</td>
<td>3</td>
<td>0</td>
<td>8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>MM</td>
<td>4</td>
<td>5</td>
<td>18</td>
<td>11</td>
<td>0</td>
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</tr>
<tr>
<td>MG</td>
<td>11</td>
<td>0</td>
<td>7</td>
<td>0</td>
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</tr>
<tr>
<td>WJ</td>
<td>2</td>
<td>7</td>
<td>3</td>
<td>25</td>
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<td>EP</td>
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<td>7</td>
<td>0</td>
<td>1</td>
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<tr>
<td>SM</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>6</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>JB</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td>12</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>JK</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Total lesions | 29 | 33 | 50 | 95 | 1 | 0

% | 26 | 29 | 45 | 99 | 1 | 0

* Taken from Colcher et al. with permission (40).
PHARMACOLOGY OF MAB GUIDED TUMOR TARGETING

Table 4 Concomitant administration of $^{111}I$-B72.3 i.p. and $^{131}I$-B72.3 i.v. (patient NJ): advantage of i.p. route

<table>
<thead>
<tr>
<th>Tissue description</th>
<th>%ID/kg</th>
<th>%ID/kg</th>
<th>RI</th>
<th>% tumor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>i.p. $^{131}I$</td>
<td>i.v. $^{131}I$</td>
<td>i.p. $^{131}I$</td>
<td>i.v. $^{131}I$</td>
</tr>
<tr>
<td>Carcinoma</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Omentum</td>
<td>44.51</td>
<td>5.85</td>
<td>7.61</td>
<td>32.6</td>
</tr>
<tr>
<td>Mesentery, sigmoid colon</td>
<td>40.04</td>
<td>5.41</td>
<td>7.39</td>
<td>29.3</td>
</tr>
<tr>
<td>Peritoneal liver capsule (n = 4)</td>
<td>39.62</td>
<td>9.19</td>
<td>5.64</td>
<td>29.0</td>
</tr>
<tr>
<td>Peritoneal gastroesophageal junction</td>
<td>39.27</td>
<td>8.45</td>
<td>4.65</td>
<td>28.8</td>
</tr>
<tr>
<td>Omentum, greater</td>
<td>11.41</td>
<td>2.75</td>
<td>4.16</td>
<td>8.4</td>
</tr>
<tr>
<td>Peritoneal peripancreas</td>
<td>45.27</td>
<td>11.30</td>
<td>4.01</td>
<td>33.2</td>
</tr>
<tr>
<td>Omentum, lesser (n = 2)</td>
<td>27.08</td>
<td>7.54</td>
<td>3.68</td>
<td>19.8</td>
</tr>
<tr>
<td>Diaphragm (n = 3)</td>
<td>27.23</td>
<td>7.76</td>
<td>3.48</td>
<td>19.9</td>
</tr>
<tr>
<td>Peritoneal spleen, capsule (n = 4)</td>
<td>29.30</td>
<td>10.72</td>
<td>2.77</td>
<td>21.5</td>
</tr>
<tr>
<td>Normal</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Adipose tissue</td>
<td>0.07</td>
<td>0.05</td>
<td>1.31</td>
<td>0.1</td>
</tr>
<tr>
<td>Mesentery, transverse colon</td>
<td>0.52</td>
<td>0.49</td>
<td>1.07</td>
<td>0.4</td>
</tr>
<tr>
<td>Peritoneum, right</td>
<td>0.84</td>
<td>0.66</td>
<td>1.26</td>
<td>0.6</td>
</tr>
<tr>
<td>Peritoneal adhesions (n = 3)</td>
<td>1.21</td>
<td>0.78</td>
<td>1.56</td>
<td>0.9</td>
</tr>
<tr>
<td>Abdominal scar</td>
<td>0.99</td>
<td>0.47</td>
<td>2.09</td>
<td>0.7</td>
</tr>
<tr>
<td>Falciform ligament</td>
<td>1.04</td>
<td>0.37</td>
<td>2.82</td>
<td>0.8</td>
</tr>
<tr>
<td>Spleen (n = 3)</td>
<td>2.70</td>
<td>3.45</td>
<td>0.78</td>
<td>2.0</td>
</tr>
</tbody>
</table>

*a* Taken from Colcher et al., with permission (40).

*b* RI was determined by dividing the %ID/kg of the tumors by the averaged %ID/kg of all normal biopsies.

as B72.3 may now be considered for use in the localization of suspected colorectal or ovarian carcinoma lesions. Radiolabeled B72.3 can also be used, along with the serum assay for the presence of TAG-72 antigen, to monitor efficacy of various therapies. These studies (40) also demonstrated that carcinoma was detected by i.p. administered $^{131}I$-labeled B72.3 but could not be detected by computer-assisted tomographic scan or other radiographic procedures in 3 of 10 patients studied; lesions as small as 1.5 cm were detected. Furthermore, efficiency of detection should improve when radionuclides, such as $^{99m}Tc$ and $^{111}In$, with more favorable imaging characteristics are substituted for $^{131}I$.

Animal Models. In view of the potential advantages of intraperitoneal tumor targeting for diagnosis and/or therapy, comparatively few studies have been undertaken to systematically compare the pharmacokinetics of clearance of administered MAb via that route in animal models. Horan Hand et al. have recently completed such a study which is reviewed here. In these studies, mice, rats, and monkeys were given $^{125}I$- or $^{131}I$-labeled MAb B72.3 i.p. and these results were compared to those of patients receiving $^{131}I$-labeled B72.3 IgG via the same route.

To determine which if any animal model is appropriate to study i.p. MAb clearance, data of individual animals were averaged for each species and compared with the data obtained from human colorectal carcinoma patients inoculated with radiolabeled MAb B72.3. Patients who received radiolabeled MAb B72.3 were those negative by gamma scan. As shown in Fig. 2, the peak of MAb in the plasma after i.p. MAb administration was approximately 3-4 h for mice and rats and 24 h for monkeys. In contrast, colorectal carcinoma patients who received MAb B72.3 showed the highest average MAb plasma levels at 24-72 h. These results suggest that the rodent may not be an appropriate model for i.p. MAb studies.

One possible explanation for the vast plasma clearance differences observed between the 3 animal models and humans may be the differences in peritoneal surface area among the species. Since it has been suggested that peritoneal surface area is directly proportional to body surface area (43), body surface area was determined for all animals and humans in this study. A strong correlation was shown to exist between body surface area and time to peak percentage of MAb injected dose in the plasma after i.p. MAb administration. Mice and rats with body surface areas of 0.78-4.89 cm² had time intervals of 2-5 h for peak MAb plasma levels. In contrast, monkeys, with an average body surface area of 32.1 cm², reached peak MAb levels at 18-24 h. Humans, with the largest average body surface area, 212 cm², and therefore the largest peritoneal surface area, had the longest time interval (24-72 h) between i.p. MAb inoculation and peak MAb plasma levels. In conclusion, results of Horan Hand et al. should be considered by investigators when designing preclinical studies using MAbs.

Studies Concerning the Effect of External Irradiation of Tumor on Localization of Radiolabeled MAb

One approach to enhance localization of MAb conjugates to tumor cells may be exposure of tumors to external irradiation prior to MAb administration. Alterations in blood flow to the tumor may increase accessibility of tumor cells to MAbs. Order et al. (44) suggested that raising the therapeutic ratio for treatment of human hepatomas was accomplished by irradiation prior to administration of radiolabeled anti-ferritin polyclonal antibody. Studies in animal models have shown a low level of augmentation of antibody binding after preirradiation of tumors. Using four different syngeneic rat hepatomas and radiolabeled anti-ferritin antibody, increases in antibody uptake in tumor versus liver after preirradiation of tumors with 1000 cGy ranged from 1.1- to 1.5-fold (45). Stickney et al. (46) have also observed a 1.5- to 2-fold enhancement of an $^{111}In$-labeled anti-p97 MAb to human xenografts pretreated with 1000 cGy of external irradiation. The mechanism for these effects was thought to be related to alterations in the tumor vasculature. Whereas the previous studies were conducted in animal models using hepatoma (45) or melanoma (46) xenografts and the clinical trials involved hepatoma patients (44), no studies have thus far been reported on the effect of preirradiation on MAb binding in colon carcinomas.

These reports led us to study the effect of external irradiation on MAb targeting of human carcinomas (47); as a model system, we used MAb B72.3 and the LS-174T human colon...
carcinoma xenograft in athymic mice. LS-174T tumors exposed to 300 cGy grew to approximately 93% the size of nonirradiated tumors, while those exposed to 600, 900, or 2000 cGy were approximately 41% the size of control tumors. Splitting the 900 cGy into three 300-cGy fractions yielded a 2-fold lower tumor volume compared with a single 900-cGy fraction. Histological evaluation of the carcinomas revealed a decrease in the number of mitoses per high power field consistent with early effects of radiation exposure. Using the avidin-biotin complex immunoperoxidase technique, carcinomas were assayed for expression of the TAG-72 antigen. No discernible variation was observed in the staining intensity among tumors in both the control and radiation treated group; i.e., differences among tumors within each group were compatible with the known heterogeneous expression of TAG-72.

Exposure of carcinomas to 300 or 900 cGy in a single fraction or 900 cGy split in three 300-cGy fractions did not yield a consistent or substantial enhanced localization of radiolabeled MAb B72.3 IgG or F(ab')2 to tumors (Table 5). A 1.5-fold augmentation of MAb binding to tumors was observed in preirradiated mice; however, these results were not statistically significant. While our results are different than those reported with other tumor models, they do not necessarily conflict. Inherent differences in tumors such as cell type of origin, size, spatial configuration, extent of vascularization, and volume of interstitial space may contribute to variability of the effect of preirradiation of tumors on antibody binding. Our results suggest that consistent augmentation of radiolabeled antibody localization to preirradiated tumors may not be a universal phenomenon.

**Recombinant/Chimeric**

Several chimeric antibodies have been generated using the variable region of murine MAb heavy and light chains with human constant regions (see the report of Morrison (48) for review). Recombinant/chimeric MAbs using variable regions from murine MAbs against human tumor associated antigens have recently been reported (49–53). For the most part these recombinant/chimeric MAbs have been generated using human γ1 heavy chain sequences and have been primarily used for *in vitro* studies of the biological activities of the recombinant/chimeric MAbs, e.g., cell mediated and complement mediated cytotoxicity (54–56). Yokoyama et al. (57) and Steplewski et al. (55) have investigated the *in vivo* utility of recombinant/chimeric MAbs to prevent tumor growth in athymic mice bearing human tumor xenografts, and have shown some utility of the γ1 and γ4 constructs. Brown et al. (58) have also investigated the *in vivo* biodistribution of a recombinant/chimeric MAb made with a MAb, B6.2 (5), generated against a human mammary tumor associated antigen.

Whittle et al. have recently generated a recombinant/chimeric MAb using the variable regions of MAb B72.3 (5) and a human γ4 and κ constant regions in COS-1 cells (53). We have studied the biodistribution of both the recombinant and recombinant/chimeric B72.3 in a murine tumor xenograft model to determine the efficacy of the IgG constructs to bind to TAG-72 and to efficiently localize human tumors *in situ* (59).

There are numerous potential advantages to the use of recombinant/chimeric immunoglobulins in clinical trials: (a) The reduction and/or elimination of the human immune response to the mouse immunoglobulin, since much of the murine immunoglobulin is now substituted with human constant region. This manipulation may also reduce or eliminate any human antiidiotype response. Several studies have demonstrated that a significant portion of patients who have been given murine MAbs develop an anti-murine immunoglobulin response (60–65). This immunological response may be reduced by the use

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Table 5 Effect of fractionated and single dose irradiation on binding of radiolabeled MAb B72.3 in athymic mice bearing LS-174T xenografts

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Control</th>
<th>3 × 300 cGy</th>
<th>900 cGy</th>
</tr>
</thead>
<tbody>
<tr>
<td>Tumor</td>
<td>10.6 ± 4.3*</td>
<td>14.6 ± 3.6</td>
<td>12.1 ± 2.4</td>
</tr>
<tr>
<td>Blood</td>
<td>6.5 ± 2.9</td>
<td>7.9 ± 2.2</td>
<td>9.3 ± 3.0</td>
</tr>
<tr>
<td>Liver</td>
<td>1.1 ± 0.4</td>
<td>1.5 ± 0.4</td>
<td>1.5 ± 0.9</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.9 ± 0.4</td>
<td>1.7 ± 1.4</td>
<td>4.8 ± 7.8</td>
</tr>
</tbody>
</table>

* Taken from Shrivastav et al. with permission (47).

* Values represent the mean ± SD of the percentage of injected dose per g of tumor and normal tissue for 7–8 mice.
of fragments of the murine MAb (64), but it should be further minimized by the use of human monoclonal antibodies. Several groups have used an alternative approach of using the variable regions of murine MAbs made against tumor associated antigens and the constant regions of various human IgGs to generate recombinant/chimeric MAbs. The recombinant/chimeric MAbs should retain the specificity of the murine MAb but they may be less immunogenic in patients since the majority of the IgG molecule contains human sequences. Preliminary studies by LoBuglio et al. (66) have demonstrated that while approximately 70% of patients who have been given murine MAb 17-1A have developed an immune response to the MAb with an antidiotypic response, only 1 of 10 patients who have been given a chimeric form of that MAb (human-γ1x) have generated an anti-17-1A idiotype response. (b) Isotype switch variants can now be engineered, since cassettes are available of all human constant regions. Thus any recombinant/chimeric MAb can now be fitted with a variety of human constant regions to define optimal pharmacokinetics, effector cell interactions, and complement mediated cytotoxicity. (c) Constructs can be engineered for alterations in MAB pharmacokinetics. Immunoglobulin domain deletions can be constructed with restriction enzyme technology to prepare smaller recombinant/chimeric constructs. Some of these may have the advantage of more rapid clearance from the body and thus one may achieve higher tumor-normal tissue MAb binding ratios and, in therapeutic studies, less marrow toxicity. Smaller constructs should also have the advantage of better penetration through the tumor mass. (d) Molecular alterations in glycosylation sites of a recombinant/chimeric Ig may also lead to more rapid body clearance of such constructs, since it has been shown previously that the glycosylation of an immunoglobulin can clearly alter its pharmacokinetics. (e) Genetic alterations in the hypervariable region have been shown to increase antibody affinity. While this is unlikely to be a common event, it should not be ignored. (f) The insertion of sequences coding for specific amino acid sequences that may function as binding sites for specific chelates, drugs, or effector cells can now also be envisioned. Thus, the parameters listed in Table 1 and those outlined in this article demonstrate that there are numerous ways to modify the pharmacology of MAB guided tumor targeting toward more efficient diagnostic and therapeutic applications.

References


Innovations That Influence the Pharmacology of Monoclonal Antibody Guided Tumor Targeting

Jeffrey Schlom, Patricia Horan Hand, John W. Greiner, et al.