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The ratio of maximum percent tumour accumulations of the pretargeting agent and the radiolabelled effector is independent of tumour size

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ABSTRACT

Our previous studies have indicated that the optimal dosage ratio of pretargeting antibody to effector is proportional to their maximum percent tumour accumulations (MPTAs). This study quantitatively describes how both MPTAs and their ratio change with tumour size, to simplify pretargeting optimisation when tumour size varies. The CC49 antibody dosages below saturation of the tumour antigen level were first examined for the LS174T tumour mouse model. Then the MPTAs of the antibody in mice bearing tumours of different sizes were determined, always at antibody dosages below antigen saturation. Historical data from this laboratory were used to collect the MPTAs of the ^{99m}Tc-cMORF effector for different tumour sizes, always at effector dosages below that required to saturate the MORF in tumour. The MPTAs versus tumour sizes for both the antibody and the effector were fitted non-linearly. The best fit of the antibody MPTA ($Y_{antibody}$) with tumour size (x) in grams was $Y_{antibody} = 19.00\ x^{-0.65}$ while that for the effector was $Y_{effector} = 4.51x^{-0.66}$. Thus, even though the MPTAs of both vary with tumour size, the ratio ($Y_{antibody}/Y_{effector}$) is a constant at 4.21. In conclusion, the MPTA ratio of the antibody to the effector was found to be constant with tumour size, an observation that will simplify pretargeting optimisation because remeasurement of the optimum dosage ratio for different tumour sizes can be avoided. Theoretical considerations also suggest that this relationship may be universal for alternative antibody/effector pairs and for different target models, but this must be experimentally confirmed.

1. Introduction

That tumour size has an influence on tumour accumulation of antibodies has been recognised for more than 20 years.[1] Although tumour accumulation in %ID/g usually decreases with increasing size,[1,2] this relationship is not without exceptions.[3,4] The tumour size influence on the accumulation of tumour targeting agent is very important both pre-clinically and clinically, because tumour size differences are common in animal tumour models and especially in patients.

Unlike conventional tumour direct-targeting with radiolabelled antibodies, optimisation of dosage and timing is more complicated in tumour pretargeting and the influence of tumour size must be considered. In conventional direct-targeting, as long as the antibody dosage does not exceed that required to saturate the tumour antigens and is nontoxic,

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any dosage may be used.[5–8] Instead of only one dosage to consider, pretargeting requires selecting two dosages and one interval (even more if imaging or clearing agents are involved). Optimisation requires that the dosages of antibody and effector be selected to provide the maximum percent tumour accumulation (MPTA) of the effector and the highest tumour/non-tumour ratios of the effector.[9]

Previously, we established experimentally that optimal pretargeting will be achieved when the dosage of antibody ($D_{antibody}$) is equal to or below that required to saturate the antigen levels in tumour and the dosage of the effector ($D_{effector}$) is just equal to that required to saturate the pretargeting antibody in tumour.[9] Under these conditions, both the antibody and effector are at their MPTAs and the tumour to normal tissue ratios are at their maximum values. For any given tumour, the optimal dosages and their MPTAs are related by the equation below, which represents the quantitative relationship under the conditions mentioned above[9] and shows that the optimal dosage ratio is proportional to the ratio of the MPTAs of the antibody to the effector.

$$\text{Optimal}\frac{D_{effector}}{D_{antibody}} = \frac{MW_{effector} \times \text{gpm} \times \text{accessibility}}{MW_{antibody}} \times \frac{MPTA_{antibody}}{MPTA_{effector}}$$

where $MW_{antibody}$ and $MW_{effector}$ are the molecular weights of the antibody and effector; gpm is the average number of the effector-binding groups on each antibody; and the accessibility is the fraction of the antibody in tumour still accessible to the effector at the time of effector administration. As the equation makes clear, the optimum dosage ratio depends upon the ratio of the MPTAs of antibody and effector. Since the two MPTAs vary differently with tumour size, selecting the optimum dosage ratio will be difficult unless it can be shown that the ratio of the MPTAs is a constant. If so, optimisation of the dosage ratio will be simplified since the optimal dosage ratio obtained from one tumour size will then be applicable to all others.

We have now examined how both the MPTAs of the MORF-CC49 antibody and labelled cMORF effector as well as their ratio vary with the size of LS174T tumours in nude mice. The pharmacokinetics of the CC49 antibody was reexamined (by assuming that the biodistribution of ^{111}In-DTPA-CC49 is sufficiently similar to that of native CC49 and MORF-CC49) to select a time post administration when tumour accumulation was essentially completed. The antibody dosage was then varied within a large range and the tumour accumulation measured at the selected time (48 h) post administration. By demonstrating a linear increase in absolute tumour accumulation with increasing antibody dosage, it was possible to select with confidence a dosage that was greatly below that required to saturate the antigen level in the tumour. Thereafter, the antibody MPTAs were all measured for dosage below saturation in mice with different size tumours. In the case of the cMORF effector, the results of multiple historical pretargeting studies from this laboratory were used to provide a series of effector MPTAs and tumour sizes at sacrifice. These data, both published and unpublished (listed in Table 1) were obtained in the same LS174T tumour mouse model administered either the MORF-CC49 or MORF-MN14 antibody. In all cases, the effector dosages were below the MORF saturating dosage established earlier. Thereafter, both the MPTAs of the antibody and effector versus tumour size were fitted and their MPTA ratio calculated.

2. Material and methods

The CC49 antibody was produced by Strategic Biosolutions (Ramona, CA) from the CC49 hybridoma. Labelling of the antibody with ^{111}In was as previously described.[4,10] The base sequences of MORF and its complement (cMORF) were as previously described.[11] The p-SCN-Benzyl-DTPA was from Macrocyclics (Dallas, TX). The P-4 resin (Bio-Gel P-4 Gel, medium) was purchased from Bio-Rad Laboratories (Hercules, CA) and the Sephadex G-100 resin was from Pharmacia Biotech (Uppsala, Sweden). The $^{111}InCl_3$ was from Perkin Elmer Life Science Inc. (Boston, MA). All other chemicals were of reagent grade and were used without purification.

2.1. *Biodistribution and tumour accumulation of ^{111}In labelled CC49*

All animal studies were performed with the approval of the Institutional Animal Care and Use Committee of UMass Medical School. For tumour induction, 10^6 LS174T colon cancer cells were injected into the left thigh of each Swiss NIH nude mouse (Taconic Farms, Germantown, NY). After injection of radiolabelled antibody, the mice were sacrificed by exsanguination via heart puncture under halothane anaesthesia. For biodistribution, samples of blood and other organs were removed, weighed, and counted in a NaI(Tl) well counter (Cobra II automatic gamma counter, Packard Instrument Company, CT) along with a standard of the injectate as previously described.[11] Blood was assumed to constitute 7% of body weight.

Three animal studies were performed. Firstly, the pharmacokinetics of ^{111}In labelled CC49 was examined in five groups ($N = 4$) of LS174T tumoured mice with tumours implanted 9 d earlier. Each animal received 30 μg (17 μCi) of ^{111}In labelled CC49 and were sacrificed at 11, 24, 48, 72 or 96 h. Secondly, the influence of antibody dosage on tumour accumulation was examined in six groups ($N = 4$) with tumours implanted 13 d earlier. Each animal received 20, 40, 80, 120, 160, or 200 μg of ^{111}In labelled CC49 (12 μCi) and was sacrificed at 48 h. Finally, the influence of tumour size on the antibody MPTA was examined in 20 mice. From day 9 to day 13 post tumour implantation, four mice per day were each administered 30 μg of ^{111}In labelled CC49 (24 μCi at day 9) and each mouse was sacrificed 48 h after injection. The antibody MPTAs were plotted individually against the tumour weight and curve fitted into a power function. As will be shown, the 200 μg dosage of CC49 did not saturate the tumours. Therefore, the convenient 30 μg dosage was selected with assurance that the tumour would not be saturated at all tumour size.

2.2. *The MPTAs of the ^{99m}Tc-cMORF effector*

In the course of multiple pretargeting studies, we have accumulated numerous pretargeting data, both published and unpublished. Only those MPTAs of the ^{99m}Tc-cMORF effector

Table 1 – Individual MPTAs (%ID/g) of ^{99m}Tc-cMORF at 3 h in mice pretargeted 48–96 h earlier with MORF-CC49 or MORF-MN14 listed along with tumour weight at necropsy. The table also lists out the gpm of MORF-antibody, its dosage and the dosage of the ^{99m}Tc labelled cMORF. (See below-mentioned references for further information.)

(a) 48 h

MPTA (%ID/g)	5.17	6.23	6.36	5.56	6.04	5.47	6.83	5.71	6.34	6.13	8.99	8.98	8.17	9.30	11.3	10.8
Tumour weight (g)	0.86	0.58	0.59	0.70	0.76	0.60	0.82	0.52	0.74	0.57	0.49	0.62	0.56	0.81	0.69	0.51
Pretargeting conditions	20 μg MORF-MN14, (gpm = 0.83), 1.05 μg ^{99m}Tc-cMORF				20 μg MORF-MN14, (gpm = 0.83), 0.87 μg ^{99m}Tc-cMORF				30 μg MORF-MN14 (gpm = 0.37), 0.40 μg ^{99m}Tc-cMORF				30 μg MORF-CC49 (gpm = 1.12), 0.87 μg ^{99m}Tc-cMORF			
Reference	Unpublished				Unpublished				Unpublished				Unpublished			
MPTA (%ID/g)	7.89	7.74	8.66	7.08	7.89	7.74	8.66	7.08	6.59	5.83	6.54	5.69	7.49	9.47	10.37	8.27
Tumour Weight (g)	0.47	0.42	0.29	0.49	0.47	0.42	0.29	0.49	0.64	0.61	0.47	0.42	0.72	0.33	0.49	0.17
Pretargeting conditions	30 μg MORF-CC49 (gpm = 0.68), 1.0–2.5 μg ^{99m}Tc-cMORF				23 μg MORF-MN14 (gpm = 0.83), 1.0 μg ^{99m}Tc-cMORF				11 μg MORF-CC49 (gpm = 1.22), 1.0 μg ^{99m}Tc-cMORF				48 μg MORF-B72.3 (gpm = 0.53), 1.0 μg ^{99m}Tc-cMORF			
Reference	10				16				16				16			
MPTA (%ID/g)	5.80	7.38	4.82	6.51	6.77	8.57	5.51	5.98	5.70	3.92	6.93	3.88	3.79	3.01	5.95	4.29
Tumour Weight (g)	1.07	0.63	1.19	0.74	0.79	0.33	0.78	1.07	0.84	1.19	0.74	0.92	1.51	1.62	0.65	0.98
Pretargeting conditions	30 μg MORF-MN14 (gpm = 0.99), 0.89–2.28 μg ^{99m}Tc-cMORF															
Reference	15															
MPTA (%ID/g)	4.66	5.70	7.00	4.08	5.65	6.43	5.95	6.29	6.60	5.41	3.04	3.04	3.46	2.81		
Tumour Weight (g)	0.74	0.51	0.40	0.95	0.52	0.46	0.71	0.56	0.55	0.78	1.55	1.63	1.66	1.64		
Pretargeting conditions	30 μg MORF-MN14 (gpm = 0.99), 0.89–2.28 μg ^{99m}Tc-cMORF				>25 μg MORF-MN14 (gpm = 1.05), 48 h, 1.61 μg ^{99m}Tc-cMORF					30 μg MORF-MN14 (gpm = 0.125.), 0.30 μg ^{99m}Tc-cMORF						
Reference	15				15					17						

(b) 72 h or 96 h

MPTA (%ID/g)	3.14	3.31	3.88	2.74	2.41	2.37	4.96	1.59
Tumour Weight (g)	1.21	1.15	1.10	1.64	1.82	1.35	0.61	2.11
Pretargeting conditions	30 μg MORF-MN14 (gpm = 0.125), 72 h, 0.30 μg ^{99m}Tc-cMORF				30 μg MORF-MN14 (gpm = 0.125), 96 h, 0.30 μg ^{99m}Tc-cMORF			
Reference	17				17			

that were measured at an effector dosage below saturation are listed in Table 1. The effector MPTAs were also plotted individually against tumour weight and the curve fitted into a power function using Excel.

3. Results

3.1. *Pharmacokinetics of ^{111}In labelled CC49*

Fig. 1 presents blood and tumour accumulations of the radiolabelled antibody over 96 h. The trends shown are consistent with the reports from other laboratories using ^{125}I or ^{111}In labelled CC49.[12–14] Tumour levels increased consistently when plotted as %ID/organ, but decreased after about 48 h when plotted as %ID/g due to tumour growth. In both %ID/organ and %ID/g, blood levels decreased consistently with time such that after about 48 h, levels are too low for further tumour accumulation.

3.2. *Tumour growth*

By measuring the size (product of width and thickness) of tumours in the pharmacokinetic study, the curve of tumour growth versus time since tumour implantation shown in Fig. 2A was obtained. Although tumour growth in this LS174T tumour animal model may vary between studies and will provide either a larger or smaller range of tumour sizes, once tumour growth becomes visible, the tumour grows roughly at 0.11 g per day. We found that the tumour weight estimated by the product of width and thickness of the tumour thigh (cm^2) is in close agreement with the actual tumour weight at sacrifice because of the largely linear relationship as shown in Fig. 2B.

3.3. *Influence of antibody dosage on its tumour accumulation*

The influence of antibody dosage on tumour accumulation was examined at 48 h since the antibody blood level thereafter is sufficiently low such that essentially no further tumour accumulation occurs (Fig. 1). Saturation of the tumour antigens will appear as a leveling of the absolute tumour accumulation (μg/g) versus dosage curve. As shown in Fig. 3, no such leveling occurs up to at least a dosage of 200 μg, suggesting that antibody accessibility to its antigen in tumour has not been compromised by any mechanism. Therefore, by definition, the percent accumulation of the antibody under these conditions is at its MPTA.

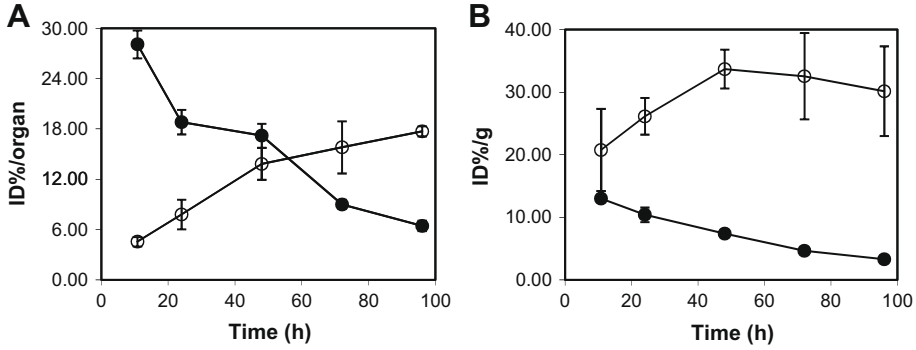

Fig. 1 – Tumour (open circles) and blood level (solid circles) versus time post antibody administration plotted as %ID/organ (panel A) and %ID/g (panel B) for ^{111}In labelled CC49 administered at 30 μg to LS174T tumoured mice. Error bars signify one standard deviation of the mean (*N* = 4).

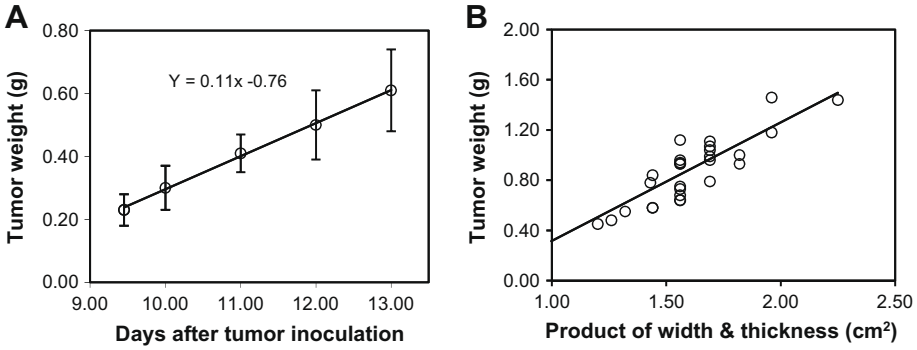

Fig. 2 – Tumour weight at sacrifice versus time since tumour implantation showing a steady increase (panel A) and a good correlation between tumour weight and the product of the width and thickness of the tumoured thigh (panel B). Error bars in panel A signify one standard deviation of the mean (*N* = 4).

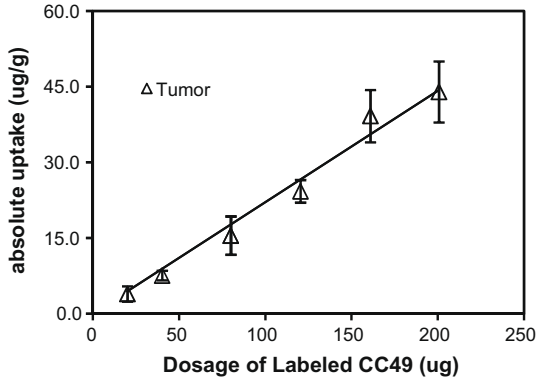

Fig. 3 – Absolute accumulations (μg/g) in tumour 48 h after intravenous administration of ^{111}In labelled CC49 at increasing dosages. Error bars signify one standard deviation of the mean (*N* = 4).

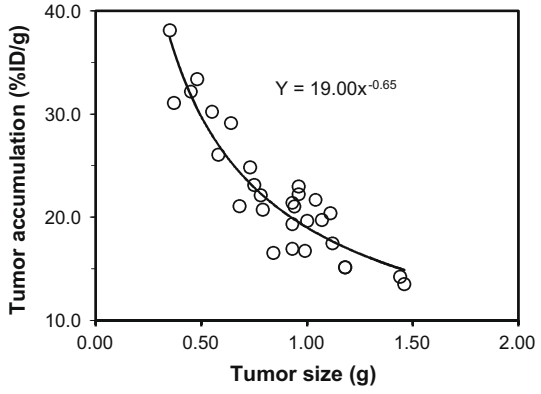

Fig. 4 – The relationship of the MPTA of CC49 antibody with tumour weight at sacrifice.

3.4. *Tumour size influence on the MPTAs of the CC49 antibody and the ^{99m}Tc-cMORF effector*

Fig. 4 shows that the antibody MPTA at 48 h decreases with tumour weight but not linearly. The best fit of these data is $Y_{antibody} = 19.00x^{-0.65}$.

The MPTA of the labelled cMORF effector in a tumour model varies with tumour size and depends on the labelled effector itself but not on the antibody.[9] While a qualitative relationship between the effector MPTA and tumour size were recognised earlier,[15] a quantitative relationship as shown in Fig. 5 has now been established using our historical data (Table 1). When the effector MPTA versus tumour weight data are fitted to a mathematical expression, the resulting function $Y_{effector} = 4.51x^{-0.66}$ has almost the identical power to that of antibody ($Y_{antibody} = 19.00x^{-0.65}$). Thus, the MPTA of the effector is proportional to the MPTA of the pretargeting antibody, resulting in an essentially constant MPTA ratio of pretargeting antibody to labelled cMORF of 4.21.

4. Discussion

We had previously shown that the MPTAs of both the antibody and the effector can be expressed as the product of

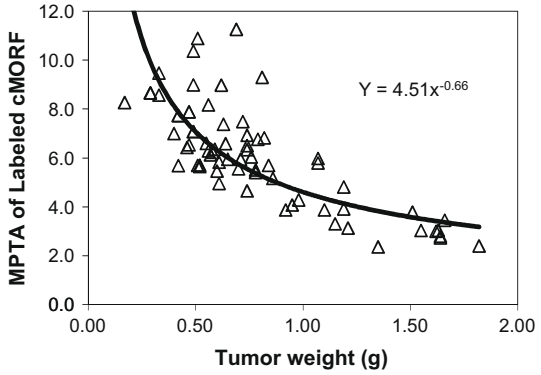

Fig. 5 – The relationship of the MPTA of the labelled cMORF effector with tumour weight at sacrifice.

the fraction of cardiac output reaching the tumour, the tumour weight, the tumour trapping fraction (*E*) and the area under the blood curve (AUC_{blood}).[9] Since the cardiac fraction and tumour weight are common to both MPTAs, the MPTA ratio of antibody to effector will be equal only to the product of the ratios of the tumour trapping fraction and the AUC_{blood}

$$\frac{MPTA_{antibody}}{MPTA_{effector}} = \frac{E_{antibody}}{E_{effector}} \times \frac{AUC_{blood,antibody}}{AUC_{blood,effector}}$$

where $AUC_{blood} = \int_{t=0}^{t=\infty} (\%ID/g)_{blood} \times dt$.

Since the AUC_{blood} ratios are independent of tumour size and we have now shown that the ratio of MPTAs is independent of tumour size, then the ratio of the tumour trapping fractions must also be independent of tumour size. It is possible that the ratio of trapping fractions may be also independent of other target changes in addition to tumour size variation such as changes in tumour type, shape, and location. Logically, any target change that affects the trafficking of antibody to tumour should also affect the trafficking of effector in the same direction.

Dosage optimisation in pretargeting is an involved procedure if accuracy is required. Optimisation always requires that the pharmacokinetics and tumour saturation dosages of the antibody and effector be determined by dosage escalation. If tumour size is now added as a variable, the process of dosage optimisation may be expected to become increasingly complex given that the MPTAs of both antibody and effector will vary with tumour size differently. Fortunately, the ratio of MPTAs is a constant of tumour size such that these measurements for other tumour sizes are no longer necessary. In addition, although experimental verification will be required, the ratio of the two MPTAs is reasonably likely to be also a constant with tumour size for other antibody/effector pairs and to be independent of tumour type, location, shape, etc. It is also reasonably likely that this ratio will be a constant for pretargeting of normal tissues such as pancreatic islet cells.

The importance of this observation may become apparent in future clinical studies. Whereas tumour size in animal models can often be controlled, that is certainly not the case in the clinic. If, as we believe likely, the MPTA ratio is a constant of tumour size in patients as in our mouse tumour model, then regardless of size, all tumours in any individual patient will be targeted with the same optimal dosage ratio.

5. Conclusion

The ratio of the MPTA for the pretargeting antibody CC49 to the MPTA for the radiolabelled cMORF does not change with the size of LS174T tumour growing in nude mice. The MPTA ratio is likely to be a constant for other pretargeting methods and other antibody/effector pairs, although this remains to be verified experimentally. This observation should simplify dosage optimisation in pretargeting studies.

Conflict of interest statement

None declared.

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