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A Semiempirical Model of Tumor Pretargeting

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Abstract

This article provides an overview of a semiempirical pretargeting model now under development. After a brief review of the pretargeting concept, the strategies available, and the complexities of optimizing the dosage and timing, a semiempirical model is described that is not only capable of optimizing dosage and timing but also capable of predicting the results of pretargeting as a function of most pretargeting variables. The model requires knowledge of the pharmacokinetics of both the pretargeting agent (usually an antibody) and the effector, the accessibility of the pretargeting antibody for the effector, and their quantitative relationships in vivo. Several misconceptions that often surround pretargeting are also clarified.

INTRODUCTION

Conventional targeting of solid tumor with radiolabeled antibodies has vastly improved recently with the development of high affinity antibodies and small antibody-like constructs. Similar improvements have also been made in parallel in the pretargeting of solid tumors both for imaging and therapy (1-10) such that encouraging results are now increasingly being reported in clinical trials of pretargeting (11). Pretargeting is popularly considered as a means of separating tumor targeting and radionuclide delivery and thus differs from conventional targeting in which the two are bound and administered together (2,3), (12-18). The concept, strategies, applications, and prospects of pretargeting have been frequently reviewed (1,2,9, 14,16-29), but the description of the pretargeting process therein remains largely qualitative and pretargeting investigations are generally performed with dosages and timing selected largely by trial and error. Recently we have made efforts to understand the pretargeting process quantitatively (30-33).

The justification for these efforts is the promise of greatly improved tumor-to-nontumor (T/NT) radioactivity ratios achieved shortly after administration of the radiolabeled effector compared to the conventional targeting with radiolabeled antibodies (34-39). By attaching the radionuclide to a small size effector designed for rapid pharmacokinetics, the nuclide not only reaches the tumor rapidly but also clears rapidly from most normal tissues. The rapidly improving T/NT ratios of the radionuclide permit early imaging and reduce unwanted radiation exposure to normal tissues. The T/NT ratios in some tissues reached in hours by pretargeting are often equivalent to those achievable in days by conventional targeting and, more favorably, the T/NT ratios by pretargeting in some other tissues such as liver and spleen may actually exceed those by conventional targeting. This latter favorable outcome will result if the antibody becomes sequestered in normal tissues but not the tumor) and thereby becomes “invisible” to the radiolabeled effector. However suggestions that pretargeting will provide higher percent

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tumor accumulation of the radiolabeled effector may not be correct (15,22,27-29), since the rapid pharmacokinetics of the effector will limit the efficiency of its delivery into tumor and thus limit the percent accumulation (40).

Since the concept, approaches, applications, and prospects of pretargeting have been adequately reviewed (1,2,9,14,16-29), there is little need for another comprehensive coverage of past studies. Instead, this contribution focuses on the difficulty of optimization in pretargeting and describes a semiempirical model under development in this laboratory that is not only capable of optimizing dosage and timing but is also capable of predicting the results of pretargeting as a function of most pretargeting variables. We begin with an introduction briefly summarizing the different pretargeting systems and conclude with a discussion of the utility of the semiempirical model. Because pretargeting has been exclusively applied to tumor as the target, this report will refer throughout to pretargeting in this context, with the understanding that in the future normal tissues as targets may benefit from pretargeting as well.

PRETARGETING SYSTEMS

At least 3 systems have now been used for pretargeting: bispecific antibody/hapten (41-42), (strept)avidin/biotin (43), and oligomer/complementary oligomer (44-46), each with several distinct strategies. The simplest strategy includes two injections and more complicated strategies may add one or more intermediate injections either to clear the pretargeting antibody in the circulation, to amplify the number of the targeting sites on the cell surface, to block the binding sites of the pretargeting antibody still in blood and normal tissues, or in the case of (strept)avidin/biotin pretargeting, to avoid the interference of endogenous biotin.

Concerning first the bispecific antibody/hapten system, the usual strategies involve two injections although a blocking or a clearing agent may be administered intermediate between the antibody and effector (47-50). Three types of hapten effectors have been reported: monovalent of moderate affinity, bivalent of moderate affinity, and monovalent of infinite affinity. Fig 1 schematically illustrates the binding patterns of the three haptens. A monovalent hapten of moderate affinity (a) was reported to provide insufficient tumor retention (51-54). As a result, bivalent haptens (b) are usually used. The rationale to the use of bivalent hapten is that bivalency has been reported to provide enhanced binding affinity to the tethered antibody on tumor compared to the untethered antibody in circulation (25). The most recent effort to enhance binding affinity (1) involves a covalent bond formed automatically following the binding of the hapten to the antibody (c). Since this covalent bond will presumably form in circulation as well, whether this strategy provides lower T/NT ratios compared to the affinity enhancement remains to be seen.

Fig 2 illustrates three streptavidin/biotin strategies. In contrast to the bispecific antibody/hapten system, the streptavidin/biotin system is more flexible since both (strept)avidin and biotin can be used as effectors. However using radiolabeled (strept)avidin as effector and a biotinylated antibody (biotin is a small molecule also known as vitamin H) as pretargeting agent (a) is no longer being pursued (43,55-56), because the slow pharmacokinetics of the radiolabeled (strept)avidin (60,61) violates the principle of pretargeting whereby the radiolabel is to be attached to an effector with rapid pharmacokinetics. Therefore the radiolabel is now attached to biotin and the (strept)avidin to the antibody (b). A potential complication of this strategy is the interference by endogenous biotin (62,63) and the immunogenicity of streptavidin (64). Avidin may be a less immunogenic substitute for streptavidin (65) possibly only because its glycosylation encourages rapid accumulation into the liver (66). While a simple two-step strategy was used initially to show proof-of-concept (67-70), most studies now include a clearing step prior to effector administration. Increasing the pretargeting interval, i.e. the time between injections, would accomplish the same goal of clearing the pretargeting antibody but

perhaps at the expense of reduced antibody expression in tumor due to internalization. Evidence has been presented that certain antibodies internalize more rapidly after conjugation with streptavidin (71,72).

Another pretargeting strategy (c) of the same system involves 3 steps, the stepwise administration of biotin-antibody, avidin, and radiolabeled biotin (43,56,73,74). The avidin serves multiple purposes: it clears the biotinylated antibody into the liver, helps clear endogenous biotin, and may modestly amplify the signal on the tumor sites by as much as a factor of three (75).

Within the oligomer/complementary oligomer system, the most extensively studied strategy thus far is to use phosphorodiamidate morpholino oligomers (MORFs). Other DNA analogues have been little used for a variety of reasons including instability of the native phosphodiester DNAs to nucleases (44,76), the high binding affinity of phosphorothioate DNAs to serum and tissue proteins (77), and the aqueous insolubility of peptide nucleic acids (PNA) depending upon base sequence (78). MORFs exhibit high hybridization affinities to their complements (cMORFs), good hydrophilicity, and fast renal clearance (79). The different MORF/cMORF pretargeting strategies now under development are shown in Fig 3, including (a) the traditional two-step pretargeting, (b) affinity enhancement pretargeting, and (c) amplification pretargeting. While investigations of the latter two strategies continue (80,81,78,82,83), emphasis thus far has been on the first (30-33,46,84-87) because of its relative simplicity.

Although not described in this article, several miscellaneous studies could be considered pretargeting such as the use of enzyme/substrate interactions as a recognition system (88) and the use of MORF/cMORF binding to quantitate the internalization of antibodies (89).

FUNDAMENTAL DIFFICULTIES IN OPTIMIZING DOSAGE AND TIMING IN PRETARGETING

Although pretargeting has improved radionuclide delivery over conventional targeting, the improvement comes with a degree of complexity not shared by conventional targeting. Compared to the two variables of conventional targeting (dosage of radiolabeled antibody and detection time), there are four dosage-and-timing-associated variables in two-step pretargeting strategies (dosage of the pretargeting antitumor antibody, the pretargeting interval, the dosage of labeled effector, and the detection time) and considerably more in three-step strategies. The relationship between the two variables in the conventional targeting is relatively simple. Provided that antigenic sites are not saturated, the percent of radioactivity accumulating in tumor does not vary greatly with the dosage of radiolabeled antibody but varies only with time. By contrast, in pretargeting, the accumulation in tumor of the radiolabel, now on the effector, will be influenced by changes in any of the pretargeting variables. Furthermore, there are at least three more system-associated variables (the pretargeting antibody, the radiolabeled effector and the tumor model) compared to only two in conventional targeting (the radiolabeled antibody and tumor model). Therefore, optimization of pretargeting is comparatively difficult especially in clinic trials (40).

In practice, optimization in pretargeting is almost always achieved experimentally. Theoretically, mathematical modeling could provide a method of simplifying the optimization. Several reports on purely mathematical modeling have appeared. For example, one report simulated an antibody/hapten pretargeting system (90), but the quantitatively predictive accuracy may have been compromised by the use of oversimplified pharmacokinetic model and assumed parameters. A similar attempt at mathematical modeling compared a biotin/streptavidin two-step pretargeting strategy to a conventional targeting but once again assumed parameters were used in the model (91). Similar observations may apply to additional reports

(92,93). In general, thus far pure mathematical modeling has not been able to predict optimal pretargeting conditions. In referring to those attempts, it has been stated that “some of the apparent anomalies between the model and experience may be the result of using assumed parameters in the model which are not truly representative” (29).

While previous reports on experimental optimization do not describe a generalized approach, two reports, both concerning the bispecific antibody/hapten two-step strategy, deserve mention. One study optimized the pretargeting interval, antibody dosage, effector dosage, and detection time (54) by considering each variable separately while holding the others fixed at arbitrary values. In the second study (94), the influence of antibody dosage on the pharmacokinetics of a bispecific antibody was first determined and an antibody dosage below antigen saturation was selected for the optimization of the pretargeting interval. Thereafter optimization of the dosages of effector and antibody was achieved by increasing the effector dosage at fixed antibody dosage and vice versa. The pretargeting interval was arbitrarily set and, as the authors stated, “this interval might require further adjustment if either the bsMAb or peptide dosage changes.”

More difficulties have been encountered in the optimization of the streptavidin/biotin pretargeting system since most of these strategies involve three-steps. Among these three-step studies, those of Sharkey et al (63) and Axworthy et al (95) did not rely solely on trial and error. In the latter study, the dosage of antibody-streptavidin was first optimized by measuring antigen saturation. Then, the streptavidin accessibility was measured by histochemical examinations of tumor at two time points. Subsequently, at a fixed antibody dosage and a fixed pretargeting interval, a dosage of the clearing agent capable of removing more than 90% of the circulating antibody-streptavidin was selected along with an optimal dosage range of the radiolabeled biotin. However, optimization is always conditional on the selection of antibody dosage and pretargeting interval. Any change in these or other variables such as different antibodies will require a corresponding change in dosage of the clearing agent and dosage of the effector. Thus these optimization studies provide limited general guidance (36,96-100).

Similar optimization studies based purely on experimental observations of the MORF/cMORF pretargeting system has encountered similar difficulties even for the simplest two-step strategy. In one example (85), the influence of pretargeting interval on the pretargeting results was first examined, followed by the influence of the pretargeting antibody dosage. It subsequently became apparent that this optimization is incomplete, resulting in incorrect conclusions about the accessibility of antibody and antigen saturation (30).

In principle, any pretargeting strategy can be fully optimized experimentally provided that sufficient studies with variations in these variables have been performed but, besides being impractical in most cases, the optimization conditions of one system established in this manner will not apply to another. Furthermore, optimization in this manner would be extremely difficult in the clinic (101-105).

QUANTITATIVE UNDERSTANDING OF PRETARGETING --- A SEMIEMPIRICAL MODEL

Recently we have made efforts to understand pretargeting quantitatively beginning with the relatively simple 2-step strategy of the MORF/cMORF pretargeting system (30-33). A semiempirical model capable of estimating the optimal tumor accumulation and optimal T/NT ratios for any combination of pretargeting variables was designed that will hopefully lead to an understanding of how each variable influences the pretargeting outcome. The influences of different pretargeting variables on both percent and absolute tumor accumulations (%ID/g and ng/g) of the effector and its radioactivity levels in normal organs were first examined so that

several quantitative relationships could be established. These relationships are summarized below.

1. Maximum percent and maximum absolute tumor accumulations (MPTA and MATA) of effector

The tumor accumulation of radiolabeled effector has been expressed quantitatively by the following equation:

$$\text{Tumor accumulation of effector } (\%ID/g) = F \times f \times W^{-1} \times \int_{t=0}^{t=\infty} E \times C(\%ID/g)_{\text{blood}} \times dt \quad (1)$$

where F is the cardiac output; f is the fraction of the cardiac output reaching tumor; W is the tumor weight; E is the tumor trapping fraction of effector (defined as the retained fraction of effector molecules reaching the tumor) and C_{Blood} is the blood level of free effector (i.e. effector in circulation not bound to circulating antibody) (33). This equation may be used to describe how the radiolabeled effector, tumor model, and pretargeting antibody influence the tumor accumulations of effector.

When the effector dosage is above that required to saturate the antibody in tumor, E will become zero beyond the saturation point, since additional effector arriving cannot be retained. However, at effector dosages below that required to saturate the antibody in tumor, E will be a constant of effector dosage since during the entire delivery process (from $t = 0$ to infinity), the same fraction of effector reaching tumor will be trapped. Therefore, under these conditions E can be placed outside the integral sign in equation (1). Furthermore, under these conditions the tumor accumulation will be a maximum in percent, i.e. the maximum percent tumor accumulation (MPTA) as shown in equation (2).

$$\text{MPTA of effector } (\%ID/g) = F \times f \times W^{-1} \times E \times \int_{t=0}^{t=\infty} C(\%ID/g)_{\text{blood}} \times dt \quad (2)$$

By equation 2, the MPTA will vary with the tumor host (via F), tumor type and size (via f , W and E) and the effector (via E and the integral of C) (33). The MPTA will not change with different antibodies provided that each antibody is not entirely inaccessible.

The influence of effector dosage on percent tumor accumulation is demonstrated schematically in Fig 4A. As shown, the percent tumor accumulation of the effector is a constant at its maximum value (MPTA) below the saturating dosage. Beyond this saturating dosage, the percent tumor accumulation gradually declines.

As shown in Fig 4B, the absolute tumor accumulation of radiolabeled effector ($\mu\text{g/g} = \%ID/g \times \text{dosage of effector } (\mu\text{g}) / 100\%$) increases linearly with the increasing dosage of effector until the saturating dosage is reached. Thereafter, the absolute tumor accumulation of the effector becomes constant at its maximum value (MATA) as further effector cannot bind to the saturated antibody localized in tumor.

2. Tumor saturating dosage of effector and accessibility of antibody in tumor

The number of moles of a monovalent effector accumulated in tumor at the point of saturation will equal the number of moles of antibody in tumor after corrected for groups per molecule

and accessibility (30,31). Thus, the tumor saturating dosage of effector ($SD_{effector}$) is related to the administered antibody dosage by equation (3):

$$\frac{SD_{effector}}{MW_{effector}} \times MPTA = \frac{D_{antibody}}{MW_{antibody}} \times \%ID/g_{antibody} \times gpm \times accessibility \quad (3)$$

Where $MW_{effector}$ is the molecular weight of the effector and $D_{antibody}$ and $MW_{antibody}$ are respectively the dosage and the molecular weight of the antibody; the $\%ID/g_{antibody}$ is the tumor accumulation of antibody; gpm is the number of the effector-binding sites on each antibody and the accessibility is the fraction of the effector-binding antibody in tumor still accessible to the effector at the time of effector administration. Since the MATA in μg is equal to the product of the number of micromoles of effector in tumor and its molecular weight, it is related to administered antibody dosage by equation (4).

$$MATA \text{ of effector} = \frac{MW_{effector}}{MW_{antibody}} \times D_{antibody} \times \%ID/g_{antibody} \times gpm \times accessibility \quad (4)$$

Unless a pretargeting antibody is radiolabeled, its tumor accumulation ($\%ID/g_{antibody}$) is difficult to measure. It is commonly assumed that the pharmacokinetics of an antibody may be accurately traced by its radioactivity if radiolabeled with a limited gpm and with care leading to preserved integrity and immunoreactivity. Although this assumption is valid in at least one case of an antibody (30), we recently found that the biodistribution of antibodies can differ significantly when labeled with the same chelator but with different linker between the antibody and the chelator (32). We therefore believe that, for the purposes of prediction, measuring the biodistribution of radiolabeled antibody should be assumed to provide only an approximate measure of the pharmacokinetics of the unlabeled antibody. Fortunately, it is not the concentration of the antibody but the concentration of the accessible antibody, i.e. the product of $\%ID/g_{antibody} \times accessibility$, that is important. This concentration of accessible antibody can be measured by observing how the tumor accumulation of the radiolabeled effector changes with increasing dosage as in Fig 4. In a recent study, we measured the accessible level of a pretargeting antibody using this effector dosage escalation approach (data not presented).

3. Quantitative presentation of effector levels in organs

Simple considerations will illustrate how the effector levels in tumor and normal tissues may be predicted (31). As mentioned above, before saturation of the antibody, the percent tumor accumulation of the effector is the MPTA. After saturation, the percent accumulation of effector in tumor will be the bound effector in tumor and the free effector present in blood within the tumor as in equation (5):

$$\text{Tumor accumulation of effector} (\%ID/g) = \frac{D_{antibody} \times \%ID/g_{antibody} \times gpm \times accessibility}{MW_{antibody}} \times \frac{MW_{effector}}{D_{effector}} + \text{free effector} \quad (5)$$

At the time of effector administration, the level of antibody in circulation is usually sufficiently low such that the antibody in blood becomes saturated with the effector. Thus the total effector level in blood is the sum of both antibody-bound and free, as expressed equation 6:

$$\%ID/g \text{ total effector in blood} = \frac{D_{\text{antibody}} \times \%ID/g \text{ antibody in blood} \times gpm}{MW_{\text{antibody}}} \times \frac{MW_{\text{effector}}}{D_{\text{effector}}} + \%ID/g \text{ free effector in blood} \quad (6)$$

In the same manner, the number of moles of effector in an organ equals the number of moles of effector bound to antibody plus the number of moles of free effector in that organ. However, we have found experimentally that the effector bound antibody in any organ other than kidney (see below) is in equilibrium with the effector bound antibody in blood (31). Therefore, these organ levels can be obtained from the antibody bound effector in blood, the organ to blood ratios of bound effector ($R_{O/B}$), and the free effector in that organ:

$$\%ID/g \text{ total effector in organ} = (\%ID/g \text{ total effector in blood} - \%ID/g \text{ total effector in blood}) \times R_{O/B} + \%ID/g \text{ total effector in organ} \quad (7)$$

Since the effector clears through kidney, the sole exception to equation 7 is this organ. The radioactivity level of effector in this organ is a function of the effector itself and is approximately independent of the accumulation of antibody.

If the pharmacokinetics of the pretargeting antibody and effector and the accessibility of the antibody are known, the biodistribution of the labeled effector under any conditions can be calculated using the above quantitative relationships (31). Since the application of these relationships requires experimental data, this model is semiempirical.

UTILITY OF THE SEMIEMPIRICAL MODEL

Besides helping to optimize dosage and timing, the semiempirical model may provide useful guidance in the selection of pretargeting strategy including the selection of antibody and effector (pretargeting pair), their evaluation in preclinical studies, and, if encouraging, their use in clinical trials. Even though empirical measurement will continue to play an important role in each step, we believe that quantitative understanding may be helpful in reducing the number of empirical measurements needed.

1. Observations useful in the optimization of timing and dosages

The following observations resulted from studies on a two-step MORF/cMORF pretargeting strategy in a given mouse tumor model will be of general guidance for the optimization of T/NT ratios and tumor accumulations in the two-step strategies of other different pretargeting systems (33).

- a. Any dosage of pretargeting antibody is acceptable that is below that required to saturate the tumor antigenic sites. Higher antibody dosages may benefit diffusion into tumor against a binding site barrier or pressure gradient but will result in increased blood levels and therefore lower T/NT ratios.
- b. Any pretargeting interval is acceptable that provide a set of acceptable T/NT ratios of antibody in all normal organs of interest. The T/NT ratios of the antibody usually improve steadily with increasing pretargeting interval over at least several days. If desired or necessary, acceptable T/NT ratios may be achieved at earlier pretargeting interval through the use of an antibody clearance agent.

- c. The optimal dosage of radiolabeled effector is the dosage just sufficient to saturate the accessible antibody in tumor.
- d. Any detection time is acceptable that provides an acceptably low level of free radiolabeled effector in circulation and tissues (except kidney) compared to the level of effector bound to pretargeting antibody.

As an example of the application of these guidelines, in the case of the two-step MORF/cMORF strategy, optimization of the dosage and timing began by separately measuring the pharmacokinetics of both the labeled pretargeting antibody and the ^{99m}Tc -labeled cMORF effector and by measuring dosage saturation of tumor by antibody. The antibody results provided an estimate of the MORF-antibody concentration in tumor and provided a means of selecting the pretargeting interval. In this case, a time was chosen when the antibody reached 1-2 %ID/g in blood. Knowledge of the pharmacokinetics of the labeled effector provided a means of selecting an acceptable detection time when the labeled effector was essentially cleared from circulation, in this case a blood level of 0.04 %ID/g at about 3 h. The accessible level of the pretargeting antibody was then accurately measured by dosage escalation of the labeled effector so that the optimal dosage of the effector could be predicted.

2. Selection of pretargeting pairs

Any comparison of different pretargeting pairs should be under optimal conditions of dosage and timing for each. Fortunately, because of the quantitative relationships provided by the semiempirical model, the experiments required to obtain the optimal conditions may not all be necessary. Using the two-step pretargeting strategy again as the example, one prediction from equation 2 is that the nature of the pretargeting antibody normally does not influence the MPTA. It has been reported correctly that the pretargeting antibody affects percent tumor accumulation of effector (36, 106-109), but these percent tumor accumulations were not at the MPTA defined by equation 2. Thus two pretargeting antibodies may differ in their level of tumor accumulation but still provide the same MPTA of the effector (33).

Selection of the antibody should therefore be dictated by the desired effector T/NT ratios rather than a desired effector MPTA. The effector T/NT ratios by pretargeting can be roughly estimated from the T/NT ratios of the radiolabeled antibody, but an accurate prediction of effector T/NT ratios requires the T/NT ratios of the accessible antibody. The tumor accessible level of antibody can be measured accurately by effector dosage escalation. Antibodies in blood may be assumed to be completely accessible to the effector. Since effector bound antibody in any organ (other than kidney) is in equilibrium with the effector bound antibody in blood, it becomes relatively easy to estimate the accessible levels of the antibody in these organs using the empirical organ/blood ratios of the accessible antibody. In this way, the effector T/NT ratios achievable with that antibody can be predicted with reasonable accuracy.

Another observation useful in the selection of the pretargeting antibody is its influence on the MATA. Higher absolute concentration of effector-binding sites will lead to higher MATA, which can be achieved by increasing the dosage of the pretargeting antibody (30), increasing the gpm of pretargeting antibody (110), choosing a pretargeting antibody with better tumor accumulation (33), or, perhaps more effectively, using an amplification mechanism (78,82, 83).

Concerning the selection of the effector, although any effector will be suitable provided it is not trapped in any normal organs, the semiempirical model shows that the MPTA of different effectors can be different and usually the effectors providing higher MPTA will be preferred. The selection among effectors can be accomplished by measuring their MPTA values by effector dosage escalation. No further studies are required. As such, judgment can be reached

on which effector is superior in tumor accumulation with no need of a pretargeting study under optimal pretargeting conditions.

3. The semiempirical model and the clinic

The ultimate objective of pretargeting optimization studies is to improve tumor targeting in the clinic. In principle, the above observations obtained from the model MORF/cMORF pretargeting studies in animals should apply equally well in patients.

The main complication in clinical translation is tumor variation. Tumors originating from the same organ may differ in antigenic expression from patient to patient and the antigenic expression of metastases may vary from its primary tumor. Experimentally, tumor type can greatly influence tumor accumulation of both antibody and effector. For example, tumor accumulation of a labeled cMORF in the LS174T tumor model (31) was 2-4 times higher than that in the CWR22 model (32). Similarly, the tumor accumulation of another labeled effector in one tumor model (111) was 10 times that in another (112). It can be easily understood from equation 2 that the MPTA of an effector may be different for different tumor models.

A better understanding of how tumor properties influence tumor accumulation of both antibody and effector would certainly be helpful both in selecting a pretargeting antibody dosage that does not saturate tumor antigenic sites and in selecting the saturating dosage of effector. Because experiences thus far on the use of different tumor models for pretargeting are limited, our understanding of the tumor influence on the pretargeting results is also limited. For example, whereas tumor size was found to correlate inversely with the effector accumulation in tumor in one tumor model (31), it was found to have no influence on the effector accumulation in another (32).

Planning a pretargeting study in cancer patient might proceed by administering the radiolabeled pretargeting antibody at a tracer dosage that is expected to be below the antigen saturation dosage. By both noninvasive imaging and blood sampling, knowledge of the pharmacokinetics of the antibody can be obtained that will help to select an acceptable pretargeting interval. Thereafter, a dosage of labeled effector that is expected to just saturate the antibody in tumor may be selected based on the assumption of an accessibility in tumor comparable to that in blood. In this way an estimate of the MPTA and the T/NT ratios can be made. Presumably a strategy of this complexity may have more relevance to tumor radiotherapy than imaging.

4. General applicability to other pretargeting systems and strategies

Thus far, the development and applications of the semiempirical model have been limited to the two-step MORF/cMORF model pretargeting system. However, the semiempirical model should apply equally well to other two-step pretargeting systems and strategies provided that the pharmacokinetic parameters are available, possibly after minor modifications. For example, a valency correction will be required in the application of equations 1-6 to the streptavidin/biotin system if multiple biotin binding sites are present. Furthermore, for any system or strategy, it will be necessary to confirm in connection with equation 7 that the accessible levels of effector-binding sites in blood and normal organs are in equilibrium. Otherwise, all quantitative relationships should remain valid.

CONCLUDING REMARKS

A semiempirical model of pretargeting is under continuous development and has been applied thus far to the two-step MORF/cMORF pretargeting strategy in mouse tumor models. The resulting observations may be used as a guide for the optimization of dosage and timing, leading to a set of optimal conditions with much less effort than required by trial and error. Even though

the application of this model to the clinic has yet to be attempted, its utility is readily apparent since the pretargeting mechanism and the quantitative relationships should be identical for patients as subjects. Nevertheless, despite these pretargeting observations, the ability to select an optimal pretargeting procedure for a patient by semiempirical analysis must await further studies on how tumor variance influences tumor accumulations of both the pretargeting antibody and the effector.

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Fig 1.

The principle of pretargeting strategies of bispecific antibody: (a) with monovalent effector of moderate affinity; (b) with divalent effector of moderate affinity, and (c) with monovalent effector of infinite affinity.

Fig 2.

The three strategies of (strept)avidin/biotin pretargeting system: (a) two-step pretargeting with biotinylated antibody and radiolabeled (strept)avidin; (b) two-step pretargeting with (strept)avidin conjugated antibody and radiolabeled biotin, and (c) three-step pretargeting with biotinylated antibody, (strept)avidin, and radiolabeled biotin.

Fig 3. Three MORF/cMORF pretargeting strategies under development. (a) Traditional two-step pretargeting, (b) affinity enhancement pretargeting, and (c) amplification pretargeting.

Fig 4. Schematic illustration of the relationship between the percent (A) and the absolute (B) tumor accumulation of effector and its dosage in pretargeting