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DEVELOPMENT OF DOSIMETRIC APPROACHES TO TREATMENT PLANNING FOR RADIOIMMUNOTHERAPY

RESEARCH ACCOMPLISHMENTS (PROGRESS)

Specific Aims

The specific aims for Quantitative Imaging/Dosimetry are:

to provide the resources and procedures for collection, management, processing, and analysis of quantitative imaging data as needed to answer the questions posed;

to generate pharmacokinetic information for radiolabeled antibodies administered under a variety of conditions (antibody amount, PEG-IL-2 pretreatment, etc.) in order to: 1) evaluate the utility of the antibody; 2) develop the optimal conditions for therapeutic use of the antibody; and, 3) provide the biologic data base required for radiation dosimetry;

to provide data on macroscopic radiation absorbed doses for all significant tissues;

to develop, test and establish software to support routine quantitative imaging and radiation dosimetry;

Quantitative Imaging

A quantitative imaging and radiation dosimetry system based upon the choice of several planar methods is in routine use by technical and professional personnel. A first priority will be to plan and implement needed enhancements to the computer programs currently serving quantitative imaging.

The present programs need to be enhanced by automating many of the operations that depend on operator choices and operator skill, care and experience. The drawing of regions of interest for the sites of uptake in organs/tumor sites, and the whole body can be semi-automated by using isocount thresholds or other improved edge finding algorithms. The main advantage of this would be reproducibility of the method and reduction of inter-operator choice on the placement and size of regions drawn around uptake sites. This is particularly important for evaluation of uptake for small structures. Additional curve fitting routines need to be developed so that the user has a choice of various curve fitting and analysis routines and can visualize and compare the graphical output functions fitted for any dose estimate. Output of programs also need to be stored in a history file to save repeating analysis.

Additional projects to be addressed during the upcoming grant period include developing a simple method that allows gamma camera images to be corrected for the resolving time of the gamma camera as noted above. With therapy doses administered in the range of 60 to 200 mCi of I-131, significant losses in count rate can occur. Further studies are required to investigate the most convenient and reasonably accurate method for overcoming this resolving time error which can lead to significant errors in quantitation and dose estimates. One solution

would be to acquire all images in live time mode. However this would require additional electronics and add to the imaging time. Two alternatives are to either inject and store a pulse outside the field of view of the gamma camera image, or use a standard source that is imaged inside the field of view of every gamma camera image.

As noted above, we have determined that a simple transmission-emission planar method can be used to provide absolute quantitation of radionuclides in large volume sources such as the liver. Since the volume can be determined with SPECT or X-ray CT, the concentration of activity in this organ can be provided for dosimetry. For smaller organs such as the spleen, kidneys, tumor sites, etc., the transmission-emission method underestimates radionuclide content because of scattered photons from background activity and other large sources in the body. A contrast recovery method needs to be evaluated as a possible way of reducing the overestimated background around small organs.

Most quantitative SPECT methods developed for Tc-99m have not been tested for I-131. Because of the high energy and significant septal penetration with I-131, correction factors must be determined that are unique for this radionuclide. A solution to subtraction of the scatter fraction events for I-131 will also be more complicated because photons greater than the upper limit of the I-131 collimator are emitted by I-131. Scatter subtraction, deconvolution and Monte Carlo methods need to be tested for I-131 SPECT.

Finally, the intent is to develop or modify existing methodology to integrate information from various tomographic imaging modalities, eg, X-ray/CT scan, and MRI.

Projects for Quantitative Planar Imaging

1.0 Volume (Mass) Determinations Using Computerized Tomography

a. Transmission Computerized Tomography (TCT)

Summation of Area Methods Transmission computerized tomography (TCT) offers an accurate means to estimate volumes *in vivo*. Most attempts at volume determination using TCT have dealt with the summation of area methods. This is one of the simpler and more practical means of calculating organ or tumor volumes using contiguous TCT slices. The accuracy of these volume determinations using the summation of area technique is in the range of $\pm 5\%$. Taking a single TCT slice, the cross sectional area of any organ of interest is automatically computed by software programs built into modern TCT units. A TCT cursor outlining the organ will give an accurate area. The volume of a specific organ then may be determined by addition of areas obtained on sequential TCT scans. Volume is thus the summation of areas times the scan widths. While most anatomical structures conform to a prolate spheroid, error in this method is caused by abrupt changes in contour of an object.

This method can also be used for determining specific tumor volume because of the predictability of tumor shape. A small tumor may be encompassed by a single cross-section. If a large tumor is evaluated, the TCT cursor is used to outline the anatomical section and contiguous TCT cuts are obtained; volume is once again determined by summation of the contiguous areas times TCT scan spacing. Accuracy for smaller tumors is affected by section sampling problems.

b. Emission Computerized Tomography (ECT)

Rollo has described a manual method and Eikman, a computer-assisted method for estimating

liver volume (mass) from planar images obtained after administration of radiopharmaceutical. DeNardo and Larson have described similar methods for the estimation of splenic weight. The accuracy of any of these methods when compared to organ weight at autopsy has been better than $\pm 5\%$. These methods require certain assumptions for organ shape. Kan and Yamamoto have used SPECT to determine liver volume. The advantage of SPECT is its independence of configuration, since no geometric assumption is necessary. All of these methods are dependent upon accumulation of radioactivity in normal tissue to define its boundaries or those of the tumor.

Using SPECT we propose to develop methods and software to determine tumor volume after the accumulation of radioactivity (labeled antibody) in the tumor itself. The mechanics and methods of calculation are straight forward and similar to those for TCT, and in routine use for determination of ventricular volumes of the heart. Edge definition can be obtained either manually or using computer programs. Non-uniformity of distribution of labeled antibody within the tumor at the microscopic level represents no problem to these gross determinations of volume. Gross non-uniformity as may occur with substantial tumor necrosis represent a potential problem affecting estimates of tumor mass (and radiation dosimetry). Since the necrosis is likely to be central, it probably will not affect edge definition. Furthermore, the volume of necrosis can also be estimated, when gross, by the same methods used for the entire tumor. Combination of TCT and ECT tumor volume estimates can be expected to provide accuracy adequate for the purposes of this project. Accurate volume measurements also provide an important objective basis for judging therapeutic efficacy.

Absolute quantitation of radioactivity in vivo is a formidable task, although the methodology has advanced remarkably in recent years. Budinger, Kuhl and Hoffman have reviewed the considerations and problems. Holman has concluded that single photon SPECT is capable of: measurement of organ or tissue volume, measurement of rate of appearance or disappearance of radiotracer, and in vivo measurement of the tracer biodistribution. Jaszcak, while appreciating the limitations of available photon flux and the resulting root mean square noise level, concluded that absolute volumes and regional radionuclide concentrations using single photon SPECT if the system characteristics are determined. Kuhl has successfully used these methods to assess regional blood flow in a more complicated structure, the brain.

2.0 Dead Time Correction of Images Recorded with the Gamma Camera:

Calculation of the radiation absorbed doses in RIT depends on accurate in vivo quantitation of radionuclide uptake in tumors and other organs that accumulate different concentrations of the radiopharmaceutical administered to a patient. Since only a small fraction of the total counts recorded from I-131 with a 1/4 or 3/8 inch thick NaI crystal are photopeak events the total count rate response of a camera for radionuclides such as Tc-99m have little value in predicting the response of the camera for I-131. Preliminary studies with a range of I-131 sources imaged in air with a Siemens 7500 gamma camera indicated that at photopeak count rates of 25,000 cps, more than half the events were lost due to the resolving time of the gamma camera.

a. Dead Time Correction of Planar Gamma Camera Images:

For a gamma camera spectrum the true count rate for the photopeak is given by:

$$N(t) = N(0)/(1 - N(0)*T)$$

where T = Resolving Time/window fraction

For I-131 and the 1/4" thick NaI detector of a typical gamma camera, the window fraction is typically only 12% of the entire spectrum when imaging a liver in a patient, so that the apparent dead time can be significantly increased compared with the true dead time. The exact value of the window fraction depends on the source depth, shape, distribution of sources in the body, etc.

A correction for dead time could be made by either acquiring all frames in live time, or using a standard source that is imaged in every frame. Live time mode acquisition can only be implemented if the total gamma ray spectrum is accessible and passed through the amplifier and single channel analyses of the camera. Another approach is to add a standard source to every camera image and compare the counts from each frame with an image of the standard alone in air. The ratio of these counts can be used to provide a correction for each frame. The true counts are obtained as follows:

$$N(t) = N(0) * N(sa)/N(sf)$$

where $N(sa)$ are the counts from the standard in air and
 $N(sf)$ are the counts detected from the standard added to the frame.

The advantage of this approach is that it measures the actual response of the detector for the photopeak events which may only be a small fraction of the total spectrum. The method is simple to use and adds no increase in time to acquisition of an image. However, the method can only be used for correction of resolving time errors if the photopeak shape and position are preserved. At high count rates, movement and distortion of the photopeak and changes in shape of the spectrum, together with smearing of events in the camera image make retrospective correction with a standard less accurate.

b. Correction of whole body scan images for dead time:

In the case of whole body scan image, correction for the resolving time cannot be made using the usual resolving time method because the count rate of the camera is changing with position over each source in the body. Since each area of the image is viewed for the "exposure" time by the moving camera. A simple solution to this problem is to place a source of known activity in the field of view of the camera over the entire scan period. A line source of constant linear activity could be imaged along the length of the whole body couch to provide this correction factor for every line of pixels along the width of the field of view. A simpler solution is to attach a source to the collimator that is outside the field of view of the whole body. The number of counts detected from this standard source can then be used to correct each line of pixels in the whole body image. A computer program could be designed that would automatically make this correction to each line of pixels, based on a correction factor derived from:

$$CF(i) = N(so)/N(si)$$

where $N(so)$ are the counts detected from the standard with no other source
 $N(st)$ are the counts from any line of pixels across the width of the patient.

A whole body scan image should be acquired with the standard attached to the collimator and no source on the whole body couch to find $N(so)$ for every line of pixels in the whole body image.

For conjugate view images, the counts from the source should be attached in some way to the collimator to permit correction for attenuation in the whole body couch.

c. Dead Time Correction for SPECT Imaging:

The projection images acquired with a rotating gamma camera can be influenced by the resolving time of the gamma camera. The reconstructed image will be modified by the changing fraction of counts lost in individual projection images. If a correction factor can be applied for each projection image, the transverse section images reconstructed from modified projection data would be intrinsically correct.

Since the depth of a source will modify the gamma ray spectrum recorded by the gamma camera, correction of SPECT data will depend on adding an image of a standard to every projection frame and retrospectively correcting the projection image data before reconstruction.

The number of counts in a individual pixel in the corrected projection image is given by:

$$P'(r,0) = P(r,0) * CF(r,0)$$

where $P'(r,0)$ is the count in the projection image at (position $(r,0)$)

$P(r,0)$ is the acquired count at $(r,0)$

and $CF(r,0)$ is the correction factor at $(r,0)$ given by the ratio of counts in air alone to the counts in each image.

Errors introduced in SPECT by neglecting the resolving time of the camera can be significant and the distortion introduced by varying count losses in each projection image may result in unpredictable artifacts in the section images. Correction for the dead time of the gamma camera in SPECT is an important problem that needs to be addressed if quantitation SPECT can be used for dosimeter estimates.

3.0 Determination of Specific Uptake of MoAb in Tumors and Organs:

When the fraction of an administered radiopharmaceutical in a tumor site or organ visualized in a gamma camera image is determined from the number of counts detected in a gamma camera image, background subtraction methods are used in an attempt to subtract the activity in tissue overlying and underlying an area of interest in the planar gamma camera image. The number of counts detected in the organ is then converted to activity and expressed as a fraction of the administered dose. The temporal pattern of counts detected is a reflection of the activity in an organ or tumor site plus a fraction that reflects the intra-organ blood volume. A method is required that can be used to determine the specific uptake or accumulation of the MoAb in the organ or tumor site since this reflects the metabolic state of the site of uptake.

The specific uptake for a radiopharmaceutical that remains in the vascular pool for a significant

time compared with the rate of specific uptake can be determined using a dual tracer method. A simpler method that depends on detecting the temporal change in activity detected in the blood pool and a selected organ or tumor site can be used if the specific uptake pattern is slow and serial blood samples can be drawn to determine the percent injected dose retention in whole blood or plasma. The method proposed relies on calibrating the gamma camera image acquired at any time post injection with the activity measured in whole blood.

Suppose the number of counts detected from the tumor at time t post injection is $N(t)$ given by:

$$N(t) = N(s) + N(b) + N(bg)$$

where $N(s)$ is specific uptake

where $N(b)$ is blood in tumor

where $N(bg)$ are background counts detected from radioactivity in superimposed tissue overlying and underlying the tumor site.

$N(bg)$ can be subtracted by choosing a suitable background region of the same thickness and count density as the immediate background area in which a tumor is visualized.

If the counts in a region of interest drawn immediately post injection are determined as $N(0)$, and the counts in a 1ml blood sample are measured at the same time, a calibration factor for any subsequent image can be derived from:

$$f(0) = N(0)/N(b)$$

where $N(b)$ are the counts per min from the whole blood sample as determined with a NaI well counter.

At any time post injection the fraction of counts in the tumor ROI can be obtained by interpolation of the blood retention data. The number of counts to be subtracted from any organ or tumor site can thus be easily subtracted to yield an estimate of the specific uptake at any time post injection.

4.0 Derivation of S Factors for RIT Dosimetry:

The MIRD S factor tables have been calculated for standard man assuming that each organ has defined volume and shape. Use of these S factors in RIT dosimetry can lead to significant errors because individual organs in patients can differ by a significant factor due to body size and disease. The spleen for example can vary by a factor of more than three compared with the volume used in standard man. Tumor volumes of difference shape and volume require S factors to calculate the estimated radiation absorbed dose.

Three methods of deriving S factors for tumors and organs in the body need to be developed and compared. The three dimensional activity and volume information provided by quantitative SPECT will be used as input for the methods planned as part of the UCDMC research program.

- a. Investigate whether simple interpolation of the S factors or absorbed fractions listed in the MIRD tables can be used to derive S factors for uptake volumes that differ from the values used for standard man.
- b. Derived S factors from convolution of a spatially invariant point source dose distribution over the three dimensional distribution of activity provided by SPECT. A fast Fourier Transform method of convolution will be used to calculate the dose distribution for macroscopically non-uniform distributions of activity which are equivalent to those that have been observed with SPECT of radiolabeled MoAb's.
- c. Evaluate improved Monte Carlo techniques that can be used to calculate the energy and dose distribution in tumors and organs of varying shape, distribution and volume.

OBJECTIVES AND APPROACH

Antibodies themselves are considered to be noncytotoxic and eliminate tumor cells by activating some host-effector mechanism(s). Previous studies suggest that the ability of these effector mechanisms to eliminate cells is limited and it appears unlikely that unmodified antibodies alone will be very useful in treating bulk disease. However, there is evidence that humoral and cellular response to some antibodies is associated with effective antitumor activity in nude mice with human xenografts and in a few human patients. Of particular importance to this program is the evidence that this biologic response may provide us with a "therapeutic window" of increased tumor blood flow and vascular permeability to enhance delivery of tumorcidal immunoconjugates.

Over the past ten years we have worked with several monoclonal antibodies, both in the development of new immunoradiopharmaceuticals for therapy as well as in the development of quantitative imaging of these pharmaceuticals in patients. Our initial work was with Lym-1 in lymphoma followed by L-6 and its human chimera (Ch-L6). We have observed evidence for in-vivo biologic activity and enhanced tumor uptake. Phase I therapy studies in lymphoma and breast cancer have demonstrated measurable tumor regression. However for breast cancer and other adenocarcinomas the therapeutic index, (particularly the tumor radiation to normal marrow radiation ratio) must be further increased to achieve an effective therapeutic approach in these non-radiosensitive neoplasms. The development of radiopharmaceuticals with these and other unique MoAb's carefully chelated with radiometals such as Cu-67 or Y-90 with shorter physical half lives, but longer effective T 1/2 in tumor tissue than radioiodines should enhance the therapeutic index and thus the therapeutic potential of this approach.

L-6 MoAb and its human chimera (Ch L-6) have provided us with the initial approach to adenocarcinomas using biologically active radioimmunoconjugate therapy for metastatic breast cancer. These antibodies are well described and have excellent cytotoxic, immunologic, and histopathologic characteristics. L-6 identifies abundant, non-shed antigen that is strongly expressed in most human carcinomas including carcinomas of the lung, breast, colon, and ovary.

Normal cells express only small amounts of this antigen. Furthermore, it mediates CDC with human complement and ADCC with human natural-killer (NK) cells and macrophages, and it inhibits the outgrowth of antigen-positive carcinoma cells in nude mice. The results of pharmacokinetic studies in patients with L-6 and Ch L-6 and evidence of measurable clinical therapeutic response is in our progress report. (Section A)

We have chosen a second MoAb, BR-96, for adenocarcinoma because of some unique characteristics which add another dimension to this therapy. BR-96, a murine IgG3, made against carcinoma line 3396, binds a Le^x-related cell surface antigen with high tumor (adenocarcinoma) selectivity, mediates ADCC and CDC and also has some direct cytotoxic effect on antigen-positive tumor cells. The human chimeric form of this MoAb (Ch BR-96) has been produced and has similar properties. Radiolabeled BR-96 (¹³¹I, ¹¹¹IN, ⁹⁰Y) has demonstrated excellent tumor uptake in our preliminary murine tumor studies.

The third MoAb chosen for this project targets the uniquely defined synthetic tumor-associated glycoconjugates (s-TAGs). 155H.7 has been evaluated in imaging studies and demonstrated breast cancer uptake uniformly and no normal tissue uptake. In our mouse models we have demonstrated good 155H.7 biodistribution with ¹²⁵I and DOTA⁸⁸Y. The long blood T1/2 at 30-40 mg. IV dose in human studies and lack of normal tissue target make it a promising addition

to our arsenal. The human chimera of this MoAb has been developed and will be available to use in a biologically active form.

Single-chain antigen-binding proteins produced by recombinant techniques have been suggested to have high potential in cancer therapy. In our opinion they provide the crucial building blocks to initiate designer molecules for antibody based radiopharmaceuticals containing the messages for tumor targeting and vascular permeability as well as radiometal chelates and effective linkage. Our previous sequencing and PCR of the Lym-1 antibody allow us to utilize these methods to create a single-chain version of this antibody. The protein produced can then be modified to improve the properties of our immunoconjugates. We have isolated and expanded the DNA. The synthetic gene encoding the single chain antibody form of Lym-1 will now be created using standard techniques to ligate restriction fragments of specific DNA. The nucleic acid linkers (EcoR1 and Bam H1) will be purchased. The O_L/P_R hybrid promoter site will be created by isolation of the two fragments from lambda DNA via restriction digest and subsequent ligation at the Hinc 2 site as shown in Figure (following section 3). Two portions of the sequence will be synthesized by machine, the segment stretching from the Dde 1 site to the Tth111 I site (encoding the ompA signal peptide with flanks), and the segment from the Ava 1 site to the Ava 2 site (encoding the peptide linker with flanks). The DNA coding for the Lym-1 light chain variable region will be obtained by Tth111 I/Ava 1 digest of PCR material already prepared from our varified PCR material, using a primer to incorporate the two stop codons on the end of the variable region message. The final product shown will then be digested with Bam H1 and EcoR1 and inserted into a pUC plasmid for selection, growth and expression in *E. coli*.

A very small group of antibodies have been found that are relatively tumor specific and are internalized into the tumor cell once they attach to the abnormal membrane molecule. There is some evidence that these target molecules may be abnormal receptors but little more is presently well known about the mechanisms of internalization. The BR-96 anti-adenocarcinoma antibody is a relatively tumor specific antibody which is quickly internalized after attaching to the cell surface of adenocarcinoma cells in vitro. Electron micrographs demonstrate intracytoplasmic distribution but further details are not known at this time. We have performed murine biodistribution studies with this antibody using In-111 BR-96 and a human breast cancer cell line in nude mice and have demonstrated excellent tumor uptake and relative low normal tissue uptake (Figure 2).

FIGURE 2

Tumor therapy in vitro and in the human breast cancer xenograph in nude mice has been initiated to study the relative therapeutic efficacy of I-131, I-125, In-111, and Y-90 in this setting compared to membrane bound MoAb therapy in the same model. Radioautography will be performed to compare localization. In-111, Cu-67, and Y-90 in these studies is being attached by DOTA 2 IT BAT and Cu-67 by TETA 2 IT BAT. Human pharmacokinetic studies will be performed with In-111 CITC BR-96 and then compared to In-111 DOTA BR-96.

Cu-67 Lym-1 has provided excellent pharmacokinetic data in the 7 patients studied to date. Furthermore as discussed in this section on major accomplishments, it has resulted in tumor regression of 30-50% volumes in the three patients receiving 5, 10 and 10 mCi respectively. Uptake was calculated from imaging data at 0.01%I.D./gram in patient 7 and verified to be 0.0125%I.D./gram by biopsy at 72 hours. This further verified the quantitative imaging approach demonstrating little or no loss of Cu-67 from the tumor during this period.

An escalating dose therapy protocol will be performed with Cu-67 Lym-1 with this DOTA linkage. However, although the radiation dosimetry is quite favorable, the high liver uptake and slow release of Cu-67 is not optimal. We plan to study the newer linkages of and changes in the macrocycle in order to diminish this hepatic uptake and long residence time.

Cu-67 and In-111/Y-90 labeled Ch L-6 and Br-96 pharmacokinetics will be compared in animals and patients. If appropriate kinetics are demonstrated on these studies then the feasibility of therapy in patients will be tested.

CCGGAATTCGGGATCTCTCTCACCTACCAACAATGCCCCCTGCAAAAAATAAATTCATATAAAAAACATACAGATAACCATC
 EcoR1 Sau3A
 OL Pr +1 SD(cro)
 TGGCGTGATAAATTATCTCTGGCGGTGTTGACTATTTTACCTCTGGCGGTGATAATGGTTGCATGTACTAAGGAGGTTGTATGG
 Hinc2 Dde 1
 ompA signal -10 -1
 Met Lys Lys Thr Ala Ile Ala Ile Ala Val Ala Leu Ala Gly Phe Ala Thr Val Ala Gln Ala
 ATG AAA AAG ACA GCT ATC GCG ATT GCA GTG GCA CTG GCT GGT TTC GCT ACC GTA GCG CAG GCC
 Lym-1 Light Variable 10 20
 Asp Ile Gln Met Thr Gln Ser Pro Ala Ser Leu Ser Ala Ser Val Gly Glu Thr Val Thr
 GAC ATC CAG ATG ACT CAG TCT CCA GCC TCC CTA TCT GCA TCT GTG GGA GAA ACT GTC ACC
 Tth111 I
 30 40
 Ile Ile Cys Arg Ala Ser Val Asn Ile Tyr Ser Tyr Leu Ala Trp Tyr Gln Gln Lys Gln
 ATC ATA TGT CGA GCA AGT GTG AAT ATT TAC AGT TAT TTA GCA TGG TAT CAG CAG AAA CAG
 50 60
 Gly Lys Ser Pro Gln Leu Leu Val Tyr Asn Ala Lys Ile Leu Ala Glu Gly Val Pro Ser
 GGA AAA TCT CCT CAG CTC CTG GTC TAT AAT GCC AAA ATC TTA GCA GAA GGT GTG CCA TCA
 70 80
 Arg Phe Ser Gly Ser Gly Ser Gly Thr Gln Phe Ser Leu Lys Ile Asn Ser Leu Gln Pro
 AGG TTC AGT GGC AGT GGA TCA GGC ACA CAG TTT TCT CTG AAG ATC AAC AGC CTG CAG CCT
 90 100
 Glu Asp Phe Gly Ser Tyr Tyr Cys Gln His His Tyr Gly Thr Phe Thr Phe Gly Ser Gly
 GAA GAT TTT GGG AGT TAT TAC TGT CAA CAT CAT TAT GGT ACA TTC ACG TTC GGC TCG GGC
 Ava 1
 Linker
 Thr Lys Leu Glu Ile Lys Gly Ser Thr Ser Gly Ser Gly Lys Ser Ser Glu Gly Lys Gly
 ACA AAG TTG GAA ATA AAA GGT TCT ACC TCT GGT TCT GGT AAA TCT TCT GAA GGT AAA GGT
 Lym-1 Heavy Variable 10 20
 Gln Val Gln Leu Lys Glu Ser Gly Pro Gly Leu Val Ala Pro Ser Gln Ser Leu Ser Ile
 CAG GTG CAG CTG AAG GAG TCA GGA CCT GGC CTG GTG GCG CCC TCA CAG AGC CTG TCC ATC
 Ava 2
 30 40
 Thr Cys Thr Ile Ser Gly Phe Ser Leu Thr Ser Tyr Gly Val His Trp Val Arg Gln Pro
 ACA TGC ACC ATC TCA GGG TTC TCA TTA ACC AGC TAT GGT GTA CAC TGG GTT CGC CAG CCT
 50 60
 Pro Gly Lys Gly Leu Glu Trp Leu Val Val Ile Trp Ser Asp Gly Ser Thr Thr Tyr Asn
 CCA GGA AAG GGT CTG GAG TGG CTG GTA GTG ATA TGG AGT GAT GGA AGC ACA ACC TAT AAT
 70 80
 Ser Ala Leu Lys Ser Arg Leu Ser Ile Ser Lys Asp Asn Ser Lys Ser Gln Val Phe Leu
 TCA GCT CTC AAA TCC AGA CTG AGC ATC AGC AAG GAC AAC TCC AAG AGC CAA GTT TTC TTA
 82 82A 82B 82C 90 97
 Lys Met Asn Ser Leu Gln Thr Asp Asp Thr Ala Ile Tyr Tyr Cys Ala Ser His Tyr Gly
 AAA ATG AAC AGT CTC CAA ACT GAT GAC ACA GCC ATA TAC TAC TGT GCC AGT CAC TAC GGT
 100 100A100B 110 113
 AGT ACC CTT GCC TTT GCT TCC TGG GGC CAC GGG ACT CTG GTC ACT GTC TCT GCA TAA TAA
 Ser Thr Leu Ala Phe Ala Ser Trp Gly His Gly Thr Leu Val Thr Val Ser Ala End End
 CGCGGATCCGCG
 Bam HI

Figure 1 The Lym-1 Single-Chain Antigen-Binding protein gene sequence. The OL/Pr promoter, ompA signal peptide, Lym-1 light and heavy chain variable regions (numbered using the Kabat system), and synthetic linker (underlined) are shown. The restriction sites used to assemble the gene are underlined and labeled.

SIGNIFICANCE

Tumor response rates greater than 70% have been achieved in forty patients with B-cell malignancies and four patients with breast adenocarcinoma resistant to conventional therapies, when ^{131}I Lym-1 or ^{131}I L-6 MoAb were used. These results were achieved in patients with advanced disease and no other options, and despite the fact that we are only now approaching the MTD for the systems. Complete remissions have uniformly occurred at the highest dose level of ^{131}I per m^2 in a small number of lymphoma patients. A variety of enhancement strategies are proposed for future exploration.

As has been stated, lymphoma patients who fail their initial therapy, have only available the prospects of decreasing responsiveness and increasing toxicity to chemotherapy. In adenocarcinoma the picture is also generally bleak. Particularly in breast cancer where one of every ten women in the United States will develop breast cancer, and of these one third will die of their disease. It is estimated that there will be 150,000 new breast cancer diagnoses, and 44,000 breast cancer deaths in 1990. Despite improved diagnostic technology and increased public awareness enabling earlier detection of the disease; despite the development of new chemotherapy agents, more aggressive regimens with use of autologous bone marrow rescue technology and higher quality radiation therapy; and despite continuous combination and recombination of available modalities of therapy there has been no change over the last 50 years in survival rate for patients who develop metastatic breast cancer. Recent attempts at treating cancer patients with monoclonal antibodies has met with variable success. In the field of breast cancer, no benefit has heretofore been demonstrated. We thus present a new approach to the treatment of metastatic breast cancer using quantitative imaging and extrapolated dosimetry to determine the likely toxicity risks and therapeutic benefits of serial radioimmunotherapy. Using this approach, regression of far advanced tumor has occurred in 4 of the first 5 patients treated. This has been accomplished with acceptable side effects.

The significance of this type of advancement in the face of these odds is remarkable. However, to be effective as clinical therapy in most metastatic cancer patients the therapeutic index of radioimmunotherapy must be further enhanced. I-131 radioactivity circulating in the marrow for several days in the marrow blood pool is the major contributor to marrow toxicity. The strategies planned in this program are expected to significantly enhance the therapeutic index of tumor to marrow and thus make these early "therapeutic successes" move into practical clinically significant therapeutic approaches for cancer therapy.

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