With financial assistance from the Department of Energy, we have shown definitively that radiolabeled antisense DNAs and other oligomers will accumulate in target cancer cells in vitro and in vivo by an antisense mechanism. We have also shown that the number of mRNA targets for our antisense oligomers in the cancer cell types that we have investigated so far is sufficient to provide and antisense image and/or radiotherapy of cancer in mice. These studies have been reported in about 10 publications. However our observation over the past several years has shown that radiolabeled antisense oligomers administered intravenously in their native and naked form will accumulate and be retained in target xenografts by an antisense mechanism but will also accumulate at high levels in normal organs such as liver, spleen and kidneys. This observation is in keeping with that of all other investigators concerned with the targeting of oligomers via intravenous administration such as those interested in antisense chemotherapy, ribozyme therapy, gene therapy and sRNA therapy. In collaboration with our colleagues at Keio University in Japan, we have investigated several commercially available vectors to improve delivery but with the consistent observation that any improvement in tumor accumulation is accompanied by a similar accumulation in normal tissues, with the results that tumor/normal tissue ratios have not been materially improved. It was this observation that convinced us that the use of radiolabeled antisense oligomers for the imaging of cancer will not be feasible until novel approaches to delivery have been developed. While awaiting these developments, this laboratory has pursued two new paths, one involving optical imaging of tumor and the other Auger radiotherapy of tumor.

Regarding optical imaging, since the major difficulty is in the poor tumor/normal tissue ratios with radioactivity, we have developed a novel method of imaging tumor in which an antisense oligomer with a fluorophore such as Cy5.5 is administered while hybridized with a shorter complementary oligomer with an inhibitor such as BHQ3 is attached. We have shown in cell culture and to a degree in tumored mice that the duplex remains intact and thus nonfluorescent until it encounters its target mRNA at which time it dissociates from its complement and the antisense oligomer binds along with its fluorophore to the target. In principal, the fluorescence should be evident only in the tumor with only autofluorescence elsewhere.

Simultaneous with the above, we have also observed, as have others, that antisense oligomers migrate rapidly and quantitatively to the nucleus upon crossing cell membranes. The Auger electron radiotherapy path results from this observation since the nuclear migration properties could be used effectively to bring and to retain in the nucleus an Auger emitting radionuclide such as 111In or 125I bound to the antisense oligomer. Since the object becomes radiotherapy rather than imaging, the delivery problem may be obviated by attaching the antisense oligomer to an antitumor antibody to improve delivery following intravenous administration. Since many antibodies are trapped in endosomes following internalization, a cell penetrating peptide such as tat will also be included to ensure transport of the complex without entrapment. Rather than covalent conjugation of the three entities, we are using streptavidin as llinker after biotinylated each component. Our recent efforts have concentrated on establishing the influence of the streptavidin linker on the properties of each component within the delivery nanoparticle. Thus, we have shown that the Herceptin antibody, when linked to a labeled oligomer via streptavidin, remains capable of directing the label oligomer to Her2+ tumor cells in vitro and Her2+ tumor xenografts in mice. In addition, we have demonstrated that a labeled antisense oligomer within the nanoparticle remains capable of migrating to the nucleus and binding to its target mRNA in vitro and in vivo. We have shown that the tat peptide also preserves its properties of cell transport when incubated as one component of the nanoparticle. Most recently, we have addressed another of our concerns, namely whether the streptavidin would adversely effect the biodistribution of the antisense oligomer. We were pleased to find that the 99mTc-labeled antisense MORF within the Herceptin three component and two component nanoparticles accumulated and was

retained in tumor in a manner suggestive of radiolabeled Herceptin itself. Thus the preserved properties within the streptavidin delivery nanoparticle of the Herceptin antibody, the tat peptide and the 111In labeled antisense MORF oligomer will explain why we have successfully demonstrated an Auger electron-mediated, antisense-mediated radiotherapy in cells in culture.

One remaining concern is that the delivery nanoparticle may deliver the Auger electron emitting radionuclide to the nucleus of normal cells as well as tumor cells. We have now performed tumored mice studies of the three component delivery nanoparticle with the antisense MORF labeled with Cy3 so that tissue slices could be examined by immunohistology for evidence of MORF accumulations in the nuclei of both tumor and normal tissues. Microscopic examination shows nuclear staining in approximately 20% of the tumor cells in animals injected with the antisense nanoparticle and 10% of the tumor cells in animals receiving the sense nanoparticle, whereas no nuclear staining is seen in the tumor cells of mice given the PBS injection as another control. No nuclear staining was observed in all sections from all normal organs.

Finally, my colleagues and I wish to express our gratitude to the DOE for their generous support of our research at a time when the NIH was unwilling to fund what they believed to be a risky