جائزة الملك فيصل العالمية
King Faisal International Prize

ARTICLES IN MEDICINE AND SCIENCE IV

THE 2003 KING FAISAL INTERNATIONAL PRIZE

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Custodian of the Two Holy Mosques
KING FAHD IBN ABD AL-AZIZ AL-SAUD
Patron of King Faisal Foundation

Since its inception, Islam has stressed the importance of knowledge and thought; hence the great encouragement and honour that scholars in Muslim countries have enjoyed over the centuries. Therefore, when the King Faisal Foundation enhanced its activities by establishing the King Faisal International Prize, it was following a well-established Islamic tradition.

It is my hope that such activities spread throughout the Arab and Islamic worlds and that these countries unite in order to realize the highest scientific and intellectual objectives.

Custodian of the Two Holy Mosques
King Fahd bin Abdul Aziz

(From King Fahd's address at the second annual ceremony of the King Faisal International Prize, 12 February 1980)

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INTRODUCTION

The King Faisal Foundation continues the traditions of Arabic and Islamic philanthropy, as they were revitalized in modem times by King Faisal. The life and work of the late King Faisal bin Abd Al-Aziz, son of Saudi Arabia's founder and the Kingdom's third monarch, were commemorated by his eight sons through the establishment of the Foundation in 1976, the year following his death. Of the many philanthropic activities of the Foundation, the inception of King Faisal International Prizes for Medicine in 1981 and for Science in 1982 will be of particular interest to the reader of this book. These prizes were modeled on prizes for Service to Islam, Islamic Studies and Arabic Literature which were established in 1977. At present, the Prize in each of the five categories consists of a certificate summarizing the laureate's work that is hand-written in Diwani calligraphy; a commemorative 24-carat, 200 gram gold medal, uniquely cast for each Prize and bearing the likeness of the late King Faisal; and a cash endowment of SR750,000 (UD$200,000). Co-winners in any category share the monetary award. The Prizes are awarded during a ceremony in Riyadh, Saudi Arabia, under the auspices of the Custodian of the Two Holy Mosques, the King of Saudi Arabia.

Nominations for the Prizes are accepted from academic institutions, research centers, professional organizations and other learned circles worldwide. After preselection by expert reviewers, the. Short-listed works are submitted for further, detailed evaluation by carefully selected international referees. Autonomous, international specialist selection committees are then convened at the headquarters of the King Faisal Foundation in Riyadh each year in January to make the final decisions. The selections are based solely on

merit, earning the King Faisal International Prize the distinction of being among the most prestigious of international awards to physicians and scientists who have made exceptionally outstanding advances which benefit all of humanity.

(Excerpt from Introduction to 'Articles in Medicine and Science 1"
by H.R.H. Khaled Al Faisal,
Chairman of the Prize Board and
Director General of King Faisal Foundation)

2003 Prize Awards in Medicine and Science

The 2003 awards were presented in March 2003

The Prize for Medicine (Breast Cancer) was shared by Professor Umberto Veronesi of Italy and Professor Axel Ullrich of Germany.

Professor Veronesi has pioneered a revolution in the management of breast cancer. His pivotal role in demonstrating the safety of a wide resection followed by radiotherapy spared countless numbers of women from mastectomy and its consequences.

Professor Ullrich is widely regarded for his outstanding contributions to the study of the molecular biology of breast cancer. Over the last 20 years, Ullrich and his colleagues have demonstrated the role of tyrosine kinase receptors as growth promoters for cancerous cells.

The Prize for Science (Chemistry) was shared by Professor M. Frederick Hawthorne of USA and Professor Koji Nakanishi of Japan.

Professor Hawthorne is one of the most creative and productive chemists in the world. His research extends over many fields, ranging from the syntheses of new compounds to novel therapies for cancer.

Professor Nakanishi's research in biologically active natural products has exceptional scientific and economic value. He has established the properties and elucidated the structures of many chemical compounds including antibiotics, carcinogenic materials, and anticancer products.

WINNERS OF THE 2003
KING FAISAL INTERNATIONAL PRIZE
FOR MEDICINE

Medal: King Faisal International Prize for Medicine

PROFESSOR UMBERTO VERONESI

Co-Winner of the 2003 King Faisal International Prize for Medicine

Professor Umberto Veronesi receives his prize from HRH Prince Sultan Ibn Abd Al-Aziz, *Second Deputy Primier, Minister of Defence and Aviation and Inspector General*

(Left: HRH Prince Khaled Al-Faisal ibn Abd Al-Aziz)

Synopsis of Achievements:

Professor Veronesi has dedicated most of his professional life to exploring the paths of research that lead to treating and improving the quality of life for cancer patients, particularly breast cancer patients. One of his major achievements has been the discovery that in small breast cancers, a simple quadrantectomy, followed by axillary dissection and radiotherapy may be as safe as a 'total mastectomy. This operation, which' proved to be valid and safe, obviated the need for the mutilating procedure of total mastectomy in over one million women around the world.

Professor Veronesi has also been involved in the "sentinel node" technique, which helps to determine the state of the axillary nodes; thus avoiding the dissection of non-invaded nodes and subsequent complications like lymphedema.

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I am very honoured to have been awarded the King Feisal Price as a sign of esteem and appreciation for my long activity in Breast Cancer Research. Breast cancer is one of the most common disease in women. There are one million cases every years worldwide and it is the first cause of mortality in women between the ages of 35 and 65 in Europe.

If I had to summarize in a few words the new results and new information in various fields, I would say that chemoprevention probably is the most important issue in the prevention area at this moment. In the area of detection, the imaging revolution has led to very high rates of nonpalpable carcinomas, causing us to redesign our approaches to these very small tumors. Regarding treatment, I would say that surgery and radiotherapy are becoming less and less aggressive and more targeted, and certainly medical treatment is more effective, especially with the introduction of new biologic and biomolecular approaches.

Chemoprevention

In the Fisher's study,[1] the use of tamoxifen and placebo in high-risk women was compared. A 50% reduction in the incidence of both invasive cancer and in situ carcinoma was observed with tamoxifen. The Milan study began in 1993, with the same drugs, aims, and duration of treatment, 5 years, but the difference was that we enrolled only hysterectomized women because we were afraid of the risk of cancer of the endometrium. [2,3]

The 10-year follow-up of these patients shows that the tamoxifen group is protected to a lesser extent than in the American study. In our opinion this is likely because some of these women had oophorectomy. One thing emerged clearly in a subgroup of 1,500 patients who were using hormone replacement therapy (HRT) along with tamoxifen: we observed a very strong protective action of tamoxifen. In this subset, the difference in the Italian study is higher than in the American study, some 70% reduction.
Of course, we need more time and we need more patients, and we have in progress a new trial limited to women on HRT These women are happy for the treatment because the beneficial effects are very important, but they take these hormones with anxiety because of the increased risk of breast cancer. It would be ideal to decrease the risk by giving them tamoxifen at low doses together with HRT.

We have good reasons to believe that in this context, a limited dose of tamoxifen *(5* mg per day) will have the same effect as a higher dose without increasing the risk of endometrial cancer.

A few words about a second prevention trial we started 17 years ago, in *1985.* Fenretinide is a derivative of retinoic acid. It has been shown to be very effective in mammary tumors in rats, as a protective agent. So we thought we could have a very simple, two-arm trial, taking patients who already had breast cancer, but with very good prognoses. We divided them into two groups, randomizing between fenretinide, for *5* years, and observation. The end point was the incidence of contralateral breast carcinomas. The results after the 10-year follow-up show a different protective activity of the fenretinide according to menopausal status. In premenopausal women, the benefit of fenretinide is considerable, a 30% to *35%* reduction in the incidence of contralateral cancer. But no benefit was seen in post-menopausal women, and the same was true regarding ipsilateral breast recurrences.

A number of ipsilateral recurrences are probably new primary carcinomas. So we expect a reduction in these ipsilateral tumors also, and this appeared, in fact, in the identical proportion: some 30% in premenopause and nothing after menopause. This is an interesting study, and was the first large randomized study in chemoprevention. And because of this difference, it also shows that retinoic acid derivatives may work through some type of endocrine mechanism.

Nonpalpable tumors

This new trend is a result of one of the new concepts of the 197 Os, when we discovered that very early cancer is biologically less aggressive than more advanced tumors. In our series, for instance the incidence of grade 1 tumors decreases with increasing size. So the problem posed in the 1 970s was how to improve imaging techniques, to have large-scale, effective early screening and detection programs. The result of this new policy was that we now observe more and more tumors that are not palpable. They are discovered mainly by mammography, sometimes by ultrasonography. In our series now we have about 29% of patients with non-palpable tumors. So the problem is what to do with these patients, what type of policy, what type of operation. But identification of these tumors is not easy. The hooked wire localization has some contraindications reported in the literature.

We developed a new technique we define as ROLL, which is a radio-guided localization of these occult lesions. We introduce a few drops of human serum albumin labeled with technetium-99 through stereotactic or ultrasound guidance directly into the lesion. If the lesion is a nodule, we use ultrasound guidance, while in the case of microcalcifications, stereotactic guidance is used. We check the position of the tracer by putting a few drops of contrast medium in the fluid, so we know exactly where the tracer is,

to be sure that the position of the tumor is really well localized. Then, with the aid of a gamma detecting probe, we check through the skin where the radioactivity is present, and when we find the high acoustic signal, in this way it is very easy to reach the tumor, we take 2cm of normal margin tissue around this point. What we want at the end is not to do a biopsy, but to do a radical operation, which is a definitive one. We assess the safety of the margins through an x-ray of the specimen to verify' the lesion's centrality. We have done more than 2,000 of these ROLLS,[4] and we believe that this method should be the future method of localization.

Conservative surgery

Another concept of the 1 970s that was very clear was that the prognosis of breast cancer depended on the presence or absence of occult distant metastases. This is the real discriminating factor. The extent of local treatment is not very important; this was clearly evident even 30 years ago. And the result of this concept went in two directions: first, it was useless to have a very extensive, mutilating operation, and breast conservation was the type of new approach. At the same time, in Milan we began introducing postoperative systemic treatments as a possible solution for destroying occult distant foci. So at the beginning of the 1 970s, we began this breast conservation program, which, in phase 1, was just conservation of the breast, but then at phase 2, the new objective was to avoid axillary dissection in node-negative patients.

In 1968 I presented to the World Health Organization in Geneva a proposal for a clinical trial on breast conservation saying that the prognosis of breast cancer depends chiefly on the development of distant metastases and, only to a very minor degree, on local treatment, with its severe and mostly unnecessary mutilations. I wrote about this exactly 35 years ago and this is what I would write today.

Regarding conservation of the breast, we had to decide which type of operation was best, and we decided that a lobectomy would be the treatment of choice. There are some 12 or 14 lobes in the breast, and we believe that to remove the entire lobe is the best way to assure a radical operation: local, conservative, but radical. I was a pathologist before I became a surgeon, so I was probably influenced by my obsession about the intraductal spread of cancer. I was convinced that very often cancer cells spread through the ducts. So this is why I decided 30 years ago to use the option of lobectomy. Lobectomy was the name I suggested, but then I changed it to quadrantectomy, because when I spoke of lobectomy to the surgeons they didn't understand what I meant. So quadrantectomy was much easier to understand as a type of surgical procedure. What we found very often inside the single lobe, inside the single ductal tree, is that there were multifocal foci of cancer, mostly intraductal, but sometimes invasive foci. This was shown very clearly by Holland in his studies.[5]

So in the 1 970s we began a trial comparing the Halsted mastectomy with what we called a quadrantectomy, plus radical radiotherapy and complete axillary dissection. The results after 20 years showed absolutely no difference in survival. It was an extensive trial with 701 patients, equally divided into two groups. And if somebody still has some doubts about the safety of breast conservation, the long-term follow-up of this study provides reassurance. The study was first published in July 1981, in the New England Journal of Medicine.[6] It had a tremendous impact on the population and also the New York Times put our results on the front page, as did the Washington Post and many other newspapers in the world. Then we decided to start new trials in the same direction, first, to see if quadrantectomy, which is quite an extensive local operation, could be reduced to a smaller one, like a simple tumorectomy, removing the tumor and giving radiotherapy as the main treatment. The local recurrences were much higher in tumorectomy patients than in

quadrantectomy patients. So if you reduce the extent of the operation, you must be prepared for an increased number of local recurrences. This may be discussed with the woman and the woman may choose to have a very limited operation and have an increased risk, which is somewhere around 20%, compared with the usual 6% in the quadrantectomy and radiotherapy.

What was surprising was that survival was not affected. Survival is linked with the presence or absence of distant occult metastases. The recurrence occurs because of some anatomic reasons, a margin that's too narrow, the presence of multifocality, extensive intraductal spread, or perhaps is a new primary carcinoma. These are anatomic reasons for the failure. But distant metastases are biological: it is the intrinsic property of cancer cells to disseminate in distant organs, as a result of a mutation in the DNA.

At a certain point a new mutation occurs and cancer cells develop this ability to disseminate. We then began to see if radiotherapy was necessary. We decided that perhaps quadrantectomy alone would be enough. So, again we randomized 567 patients into two groups, quadrantectomy and quadrantectomy plus radiotherapy. Local failures were much higher in the quadrantectomy, nearly three times more, but, again, survival was not affected.[7]

During these trials we discovered a couple of things. In trial number 2 (tumorectomy and radiotherapy), we looked carefully at the rate of local failures according to resection margins, and we found there was no difference. With positive margins or negative margins, the rates of recurrence are not different, explained by the fact that radiotherapy will likely level off all these possible differences. So if you use radiotherapy, the problem with margins is not so important.

Another finding of some interest was in the Milan 3 trial, where we compared quadrantectomy alone and quadrantectomy with radiotherapy. After quadrantectomy

alone, there is a difference in recurrence rates according to age. Young women have a very high risk of local recurrence if you don't use radiotherapy, but this rate will decrease with increase in age, and after *55* it is reduced to 10%, and after *65,* there is no difference in local recurrence compared with the group who received the radiotherapy. So it is clear that radiotherapy is absolutely essential before menopause to achieve an acceptable rate of local recurrence, but with increasing age, probably after *65,* one might avoid radiotherapy.

So what we learned from these studies in Milan is certainly that conservative treatment offers equal curability rates to total mastectomy with better aesthetic outcomes and quality of life; that local recurrences do not influence prognosis; that resection margin positivity does not influence recurrence rates; and that women older than 65 may avoid radiotherapy.

Studying axillary dissection was the second objective of our study. We definitely knew that regional node dissection, by itself, did not improve survival, shown by two very important studies. One was Bernard Fisher's study published in 1 985[8] comparing total mastectomy with simultaneous axillary dissection with a total mastectomy without dissection, performing a delayed dissection only in case of the appearance of axillary metastasis. The final survival was not changed. During this time period we did the same type of operation on the internal mammary chain. Seven hundred patients were divided into two groups. One group had a mastectomy plus axillary dissection and internal mammary dissection. The other one had just the Halsted operation. After 30 years of follow-up, there is absolutely no difference in survival. There are, of course, pros and cons. In favor of axillary dissection is the information on status of the axillary nodes, which is very important. This is the most important prognostic factor, and this information may improve adjuvant treatments in principle. But against it is the fact that the removal of healthy axillary lymph nodes is certainly useless

and also may be dangerous, because this is immuno-competent tissue, and I think it is not an intelligent measure to remove this defense-type tissue.

It is important to know that the spread of cancer cells from the breast to the axilla would be a regular spread, an orderly distribution. And, in fact, this was a study I did 10 years ago on 1,224 patients, carefully evaluated one by one, targeting all the lymph nodes, and we discovered that in *54%* only the first level was involved, in 23% the first and the second levels were involved, and in 21% the first, the second, and the third levels were involved. So, 98% of the patients had a very regular, orderly distribution and spread of cancer cells in the breast from the first to the third levels. Less than 2%, had so-called skip metastases, i.e. nodes in the third level and in the second level, involved without involvement of the first level.

With this information, we decided to go for the sentinel node. The sentinel node is the first lymph node that drains the region where the neoplasia is located. We began studying the sentinel node procedure after the experience by Morton[9] in melanoma, and in breast cancer by Armando Giuliano.[10] To localize the sentinel node we decided to use human colloidal albumin labelled with technetium-99. We put a limited amount of this tracer close to the tumor, or in the subcutaneous area. A few minutes after injection, you could lymphoscintigraphically see the lymph ducts, and their distribution. After a couple of hours, intermediate radioactivity disappears and only the lymph node is present. So then we mark the lymph node position on the skin to make it easy for the surgeon to remove the sentinel lymph node. This is a very simple operation with a probe. The gamma probe is guiding the surgeon, and the lymph node is removed in no more than 10 minutes.

We began the sentinel node procedure systematically in 1995. Now we have had more than 4,000 patients with breast cancer treated with sentinel node biopsy.[11] Sentinel

node biopsy and simultaneous axillary dissection to prove the concordance were performed in 371 patients. Randomized trial comparing the total axillary dissection versus the sentinel node policy was carried out in 516 patients. Routine sentinel node biopsy is done now for all our patients, and no dissection was performed in more than 2,000 patients.

The method used to investigate the removed sentinel node is of the utmost importance because the decision to forego axillary dissection or to remove the axillary nodes will be taken while the patient is under general anesthesia for removal of the primary tumor. In our experience 17% of sentinel nodes found negative by intraoperarive frozen section analysis proved to be metastatic on definitive histological analysis. So we developed a new, more extensive intraoperative frozen section method. This has now been perfected, and it involves bisecting the removed node along its major axis and embedding and freezing both halves. Fifteen pairs of adjacent sections, 4 gm thick, are then cut at 5O-~im intervals in each half node, amounting to 60 sections per node. Whenever residual tissue is left, additional pairs of sections are recut at 100- jim intervals, until the node is completely sampled. One section of each pair is routinely stained with hematoxylin and eosin and examined. As soon as malignant cells are found, the examination stops because the result is positive. If there is any doubt about the presence of malignant cells in a given section, the other section is immunostained for cytokeratins, using a rapid method with monoclonal antibodies.

So, for sentinel node biopsy, intraoperative frozen section examination has become the definitive histologic examination at our institute, and it is the way the surgeon receives adequate information to decide whether to perform complete axillary lymphadenectomy during the operation on the primary. Some centers now use a polymerase chain reaction for detecting micrometastases. The sensitivity and specificity of methods for detecting micrometastatic disease

in sentinel nodes are likely to improve in the near future.

We randomized exactly in two groups sentinel node biopsy and axillary dissection or sentinel node biopsy, if positive, axillary dissection; if negative, nothing. We found in positive axillae, 35% of both arms. So the ability of the sentinel node procedure to stage the axillae, to select the positive axillary node compared with the normal axillary dissection was proved. At this point we believed that we should not go on with uncertainties and introduce sentinel biopsy as a routine procedure.

Then we had another problem, that in having this type of very accurate examination of the sentinel node, very often we found very tiny metastases, so-called micrometastases, of 2mm. Did this have any role in prognosis or was this something we could overlook? In our study, we found, in the A arm, total axillary dissection, 32% positive sentinel nodes and 35% in the B arm. Of these 83 cases, one-third were micrometastases, quite a good number-34%. Of these, 82% had no metastases in other non sentinel nodes. So I would say that the single micrometastasis in the sentinel node predicted a good condition in the majority of the cases in the other lymph nodes.

We have more than 2,000 patients in the third series; if the sentinel node biopsy is negative, they will have no dissection. Of course, it is important to explain the option clearly to the patient. We provide an informed consent form that says, "I agree that in case of negative sentinel node biopsy, no axillary dissection will be performed. I am aware that there is a low risk, 3% to *5%,* that enlarged axillary nodes may appear in the future. In that case, I will be submitted to axillary dissection. For that reason, I agree to have a clinical examination every 4 months". This is a prerequisite for having this procedure done.

A few words about internal mammary nodes. Internal mammary nodes are not important for cure, but are an

important prognostic factor. The internal mammary node's prognostic power is similar to the prognostic power of the axillary involvement. We are now doing more and more internal mammary node biopsies with the sentinel node technique. It takes just takes 10 minutes more. You use the same incision used for quadrantectomy. You may retract the skin and very easily reach the second and third intercostal spaces. This procedure leads to what we call stage migration, because the more accurate histology of axillary sentinel nodes, which I explained with the 60 sections, and the histologic examination of the internal mammary nodes, will modify the stage distribution of breast cancer patients. In our previous trials we found 27% histologically positive axillary nodes in Ti-NO patients, but in our new trial, as I showed, we went up to 34% because of the difference in histologic examination. The better the pathologist, the higher the number of positive nodes. So we have 7% migration of cases from stage I to stage II. Of the last 300 patients in whom we did the internal mammary node biopsy, in 10% of the case the stage did change.

Finally, what we observed in our trial number 3 was that 85% of recurrences occurred in the area where the tumor was removed, in the scar area. In other quadrants the incidence was very limited. It was similar to the contralateral tumors. Radiotherapy should be limited to that area. To irradiate the whole breast is unneeded. It may perhaps prevent a second tumor, but in that case we should irradiate both breasts.

We started a new program defined as lobar radiotherapy. We decided to use intraoperative radiotherapy using a linear accelerator with a robotic arm, and an 6-cm diameter collimator directed to the area where the tumor was removed. We have now treated 350 patients. At the beginning we carried out a dose-finding study using only intraoperative radiotherapy for the boost. Then we decided to increase the dosage, to have the complete radiotherapeutic treatment of breast cancer done intraoperatively.

In 10 or 15 minutes, we give the radiation that will save women a period of 1 month or more going to radiotherapy.

Now, we have to prove it with a trial, which began at the end of 2000. It is breast-conserving surgery and postoperative external radiotherapy, which is the current standard, compared radiotherapy. We give 21 Gy, which bioiogically is identical to the *55* Gy of external radiotherapy.

It will be a tremendous change because with breast conservation, the axillary sentinel node, and the intraoperative radiotherapy in one single operation, in one hour, a patient will receive all locoregional treatment.

This is what I call a minimalistic approach to breast cancer, which, of course, must be done with great care, but, in my opinion, is likely to be the future.

SELECTED REFERENCES

1. Fisher B, Costantino JP, Wickerham DL, et al. Tamoxifen for prevention of breast cancer: report of the National Surgical Adjuvant Breast and Bowel Prolect P-I study. J Natl Cancer Inst 1998;90: 1371-1388.

2. Veronesi U, Maisonneuve P, Costa A, et al. Prevention of breast cancer with tamoxifen: preliminary findings from the Italian randomised trial among by sterectomised women. Italian Tamoxifen Prevention Study. Lancet 1998;352:93-97.

3. Veronesi U, De Palo G, Marubini E, et al. Randomized trial of fenretinide to prevent second breast malignancy in women with early beast cancer. J Natl Cancer Inst 1999;91:1847-1856.

4. Luini A, Zurrida 5, Paganelli G, et at. Comparison of radioguided excision with wire localization of occult breast lesions. Br J Surg 1999;86:522-525.

5. Holland R, Connolly JL, Gelman R, et at. The presence of an extensive intraductal component following a limited excision correlates with prominent residual disease in the remainder of the breast. J Clin Oncol 1990;8:113-118.

6. Veronesi U, Saccozzi R, Del Vecchio M, et at. Comparing radical mastectomy with quadrantectomy, axillary dissection, and radiotherapy in patients with small cancers of the breast. N Engl J Med 198 1;305:6-ll.

7. Veronesi U, Salvadori B, Luini A, et at. Breast conservation is a safe method in patients with small cancer of the breast. Long term results of three randomized trials on 1,973 patients. Eur J Cancer 1995;31:1574-1579.

8. Fisher B, Redmond C, Fisher ER, et at. Ten-year results of a randomized clinical trial comparing radical mastectomy and total mastectomy with or without resection. N Engl J Med 1985; 312:674-681.

9. Morton DL, Wen DR, Wong JH, et at. Technical details of intraoperative lymphatic mapping for early stage melanoma. Arch Surg 1992;127:392-399.

10. Giuliano AE. Sentinel lymphadenectomy in breast cancer. J Clin Oncol 1997;62:2345-2350.

11. Veronesi U, Paganelli G, Viale G. et at. Sentinel lymph node biopsy and axillary dissection in breast cancer: results in a large series. J Natl Cancer Inst 1999;91:368-373.

PROFESSOR AXEL ULLRICH

Co-Winner of the 2003 King Faisal International Prize for Medicine

Professor Axel Ullrich receives his prize from
HRH Prince Sultan Ibn Abd Al-Aziz,
Second Deputy Primier, Minister of Defence and Aviation and Inspector General

Synopsis Of Achievments

Professor Ullrich's pioneering work on the design of genornic-based target-driven drugs marks the beginning of a new era in molecular medicine. He was the first to elucidate the DNA sequence that encodes the precursor of human insulin. He was also the first to elucidate the sequence of a cell surface molecule, the EGF-receptor, which paved the way to molecular analysis of cell communication processes that are vital for life, as well as defects that are involved in the pathogenesis of major diseases such as diabetes and cancer. In 1984, his group completed the sequence of a growth regulating peptide, EGFR. That discovery provided the basis for a mechanistic understanding of the oncogene concept because it allowed for the' first time to connect a gene product of a known function (the EGFR) with an established oncogene (the v-ErbB, of the avian erythroblastosis virus). Another finding emanating from EGFR-sequencing was the discovery of an EGFR-related gene that encoded for the receptor of HER2/c-erbB2; that ligand was later found to be the human homolog of a mouse neuroblastoma oncogene. Prompted by these results, Ullrich, in collaboration with D. Slammon at UCLA, carried out genomic analysis of primary breast tumors, which revealed that the gene encoding for HER2 was over-expressed in 25% of all breast cancers and that disease progression was significantly higher in patients with amplified HER2 gene than those with a normal HER2 genotype in their breast cancer cells. That pivotal discovery provided the first genetic prognosis determinant for a major type of human cancer. Ullrich and his team then raised a series of monoclonal antibodies (MABs) against HER2, one of which (4D5 MAB) was found to inhibit the growth of HER2-overexpressing breast cancer cells. 4D5 MAB was therefore

humanized and developed into a therapeutic agent – Herceptin - for treatment of metastatic breast cancer. This is the first anti-oncogene therapy for a human cancer. Another MAB (2D4) generated in Ullrich's laboratory is currently being developed into a second-generation therapy for HER2-overexpressing breast cancer tissue.

Signal Transduction Pathways as Intervention Targets for Novel Cancer Therapies

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If a team is to function smoothly, its members must be able to communicate effectively. This is true also for a multicellular organism, such as the human body which consists of 10^{14} individual cells. These cells are equipped with a sophisticated communication system which they use to receive signals from other cells and to transmit these into their interior by means of specific antenna molecules (receptors) on the cell surface. By operating various molecular switches, this system regulates many important biological processes, such as cell growth, -metabolism, -survival, tissue regeneration, or the specialization of immature cells into stomach, skin or blood cells.

However, the molecules participating in these signal cascades also contribute greatly to diseases, such as cancer, either because they are defective or because they are produced in the wrong quantities at the wrong time or in the wrong place. Indeed, the products of all "cancer genes" so far known to exist in man are anomalous variants of endogenous growth factors, growth factor receptors, or components of receptor-mediated signal transduction pathways.

Over the past 30 years, innovative laboratory research has led to an enormous expansion in our understanding of cellular biology and genetics. Using this knowledge, we have learnt about the molecular basis of disease and, concomitantly, a new approach to medicine

has emerged that targets the genes, proteins or processes that are pivotal to the development and/or progression of disease. Nowhere is this more evident than oncology, where understanding some of the genetic and biological abnormalities that are responsible for malignant transformation and tumor growth has enabled the development of therapies that specifically target malignant cells. Today, we are beginning to realize the benefits of these new target-specific therapies. The first agents to be licensed for clinical use, such as Herceptin ™, MabThera™ and Glivec™, are now commonly available used to treat certain tumors.

Since 1950, intense efforts have focused on the development of chemotherapeutic drugs for cancer treatment. However, in the early 1970s it became clear that this approach was unlikely to cure the majority of tumors.

Therefore despite the efforts of scientists and clinicians, it became clear that a new approach to cancer treatment was needed. This realization coincided with the development of a revolutionary new technology termed "gene technology" or "recombinant DNA research". This technique provided the tools that enabled us to understand the fundamental mechanisms that control the function of normal cells, and the changes responsible for many diseases. Already in the mid 1970s, with the use of this new technology came the expectation that we would discover new ways to treat various diseases, including cancer. More than 25 years later, we are beginning to realize that expectation.

Using gene technology, we have identified many of the genetic changes that are responsible for malignant transformation and tumor growth. For example, we have learnt about oncogenes, tumor-suppressor genes, signal-transduction pathways, which are essential for biological communication and many other genes, which, when they behave abnormally, have tumorigenic effects, e.g. genes

that control cellular proliferation, invasion, adhesion, apoptosis and differentiation. We also understand how extracellular factors can act at the cell surface to generate messages that can initiate genetic changes. Receptors and signal transduction molecules are pivotal in these processes. Using this knowledge, we have identified critical genes or proteins in tumor cells that, if we can inactivate them, may inhibit tumor growth. These genes and proteins are potential targets for the development of novel anticancer agents (fig. 1).

A plethora of potential targets for cancer therapy have been identified and, in the future, we will probably discover many more. At present, we do not have enough knowledge to define the exact characteristics of a "good" therapeutic target. However, it is reasonable to assume that targets should be essential for tumor cell function and present in a significant proportion of patients, but they should not be essential for normal function. Agents that inhibit targets that meet these criteria should, theoretically, inhibit tumor cell activity and have low toxicity. Considering our, albeit limited, clinical experience so far, it is clear that target-based agents can be both efficacious and safe [1], e.g. Herceptin [2] and MabThera [3].

In the early 1980s, the first members of a family of genes, the kinases, were cloned. Kinases play an important role in signalling pathways. Among the kinases there is a sub-group commonly known as receptor tyrosine kinases (RTKs). RTKs are involved in the initiation of cellular activation and signalling in response to extracellular stimuli.

The kinase family includes over 500 genes, and many of them function abnormally, or are dysregulated, in tumor cells. The RTKs form an important sub-family of about 60 cell surface proteins. The first RTKs to be cloned were the epidermal growth factor receptor, EGFR [4], HER2 [5], insulin receptor [6], insulin-like growth factor receptor [7], platelet-derived growth factor receptor □ [8], and Kit [9].

Studies in normal cells showed that RTKs control important processes, such as proliferation, migration and survival. However, certain RTKs are frequently over produced by cancer cells, resulting in up-regulated activity, which can promote tumor development and tumor cell growth, invasion and metastasis. The causal involvement of RTK abnormalities in the development and progression of cancer, and other diseases, makes them excellent targets for therapy (table 1). Currently at the forefront of target-specific anticancer therapy are agents directed against the EGFR and HER2.

The EGFR was discovered in the early 1980s and was the first molecule to be identified as a potential target for anti-cancer therapy [10]. This RTK is known to be an important regulator of proliferation and regeneration in normal tissues. However, as we know today, many solid tumors abnormally overproduce the EGFR. In 1984, the complete cDNA was cloned from an EGFR-overexpressing cervical carcinoma cell line [4]. After the sequence was elucidated, the EGFR was recognized as the human homolog of the avian oncogene v-*erb*B, which is the tumorigenic element of the avian erythroblastosis virus [11]. Therefore, EGFR *erb*B was the first example of how a normal gene can be converted into an oncogene.

Subsequently, numerous studies have shown that EGFR overexpression is involved in the development and progression of many types of cancer [12-14]. More recently, research has demonstrated that the EGFR acts as a point of integration for signals arising from other receptors and pathways, e.g. G-protein-coupled receptors and cytokine receptors. These cross-talk mechanisms mean that a wide range of stimuli, including physiological, neurological and environmental factors, can activate the EGFR. These interactions are intriguing, as they represent a mechanism by which numerous physiological factors can affect the progression of cancer.

During the attempts to clone the EGFR gene in the mid 1980s, another gene was accidentally cloned, HER2 (human epidermal growth factor receptor-2). The HER-2 gene, also called c-*erb*B-2, was later recognized to be a human homologue of the rat *neu* oncogene. HER2 is closely related to the EGFR and the receptors can cross communicate, enabling them to act together in transmitting signals.

In 1987, research work carried out with the clinical oncologist Dennis Slamon (UCLA) demonstrated that, amplification of the HER2 gene was linked to disease progression in patients with breast cancer [15]. Moreover, a HER2-positive tumor status was reported to correlate with poor breast cancer prognosis, including reduced relapse-free and overall survival [15-18]. The association between HER2 and breast cancer progression resulted in HER2 being used as a target for the development of new cancer treatments. Against this background, several anti-HER2 monoclonal antibodies (MAb) were developed by my research group at Genentech, one of which was muMAb 4D5, a murine anti-HER2 MAb [19-22]. After the antitumor activity of muMAb 4D5 was confirmed [19], its hypervariable antigen-binding regions were genetically grafted onto a human immunoglobulin framework to produce a humanized anti-HER2 MAb, Herceptin [23]. The US Food and Drug Administration approved Herceptin for the treatment of patients with metastatic breast cancer in 1998, just 13 years after the gene was first cloned. Now, Herceptin is available to breast cancer patients worldwide.

Herceptin is the first example of the transition from gene discovery to target identification and validation in solid tumors to the successful development and use of a target-specific agent. This process has been similarly executed for other gene discovery to targets, including the EGFR (and other members of the HER family), Bcr-Abl and vascular endothelial growth factor receptor. A number of target-specific agents are now available for use and even more are advanced in clinical development (table 2).

Several approaches can be used to block target activity. Anti-receptor monoclonal antibodies are a common approach, although antibodies can also be used to recruit immunological effector cells, deliver a toxic agent or inhibit ligand activity. Inhibiting RTK activity is another method; for instance, agents like Tarceva™ and Iressa™ specifically block the activity of the EGFR tyrosine kinase. Target activity can also be inhibited at a molecular level by using gene therapy to alter the expression of target genes [24]. In addition, specific cellular functions can be inhibited by using antisense oligonucleotides or siRNA to disrupt protein production [25] and ribozymes to interfere with translation [26].

Despite the relatively small number of target-specific agents that are currently available, there is great expectation that this type of agent will have a substantial impact on the way we treat cancer. When more agents are approved by regulatory agencies, clinicians will have to select the most appropriate treatment regimen for each patient. Ultimately, patients will probably receive a highly individualized regimen, comprising a cocktail of target-specific agents. The rationale for this approach is to attack the tumor through several mechanisms, thereby minimizing the likelihood of developing resistance.

In the future, treatment decisions are likely to be based on clinical information, combined with more specific data concerning tumor expression of relevant target molecules, and the genotype of the patient. This type of approach is already evident in indications where target-specific treatments are available; for instance in patients with breast cancer, screening for HER2 status is necessary to identify which patients may benefit from treatment with Herceptin. The development of this "patient-specific" approach to therapy will involve the integration of various resources to ensure that patients receive optimal care (fig. 2). However, more immediate challenges for oncologists include investigating the benefit of using these novel agents

in combination with each other and optimizing their integration into standard treatment regimens.

We expect that these novel agents will alter our perception of cancer and will hopefully change it from a terminal to a chronic disease. Treating cancer as a chronic condition is likely to place more emphasis on the long-term safety of anticancer agents and may shift cancer care toward an outpatient or nursing-based system. Fulfilling these expectations clearly relies on the success of target-based treatments. At present, our clinical experience with agents such as Herceptin, MabThera, Glivec and Tarceva suggests great promise, although there are still many barriers to overcome.

Selected References:

1 Shawver LK, Slamon D, Ullrich A: Smart drugs: tyrosine kinase inhibitors in cancer therapy. Cancer Cell 2002;1:117-123

2 Slamon DJ, Leyland-Jones B, Shak S, Fuchs H, Paton V, Bajamonde A, Fleming T Eiermann W, Wolter J, Pegram M, Baselga J, Norton L: Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. N Engl J Med 2001; 344:783-792.

3 McLaughlin P, Grillo-Lopez AJ, Link BK, Levy R, Czuczman MS, Williams ME, Heyman MR, Bence-Bruckler I, White CA, Cabanillas F, Jain V, Ho AD, Lister J, Wey K, Shen D, Dallaire BK: Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998; 16:2825-2833.

4 Ullrich A, Coussens L, Hayflick JS, Dull TJ, Gray A, Schlessinger J: Human epidermal growth factor receptor cDNA sequence and aberrant expression of the amplified gene in A431 epidermoid carcinoma cells. Nature 1984; 309:418-425.

5 Coussens L, Yang-Feng TL, Liao YC, Chen E, Gray A, McGrath J, Seeburg PH, Libermann TA, Schlessinger J, Francke U, Levinson A, Ullrich A: Tyrosine kinase receptor with extensive homology to EGF receptor shares chromosomal location with neu oncogene. Science 1985; 230:1132-1139.

6 Ullrich A, Bell JR, Chen EY, Herrera R, Petruzzelli LM, Dull TJ, Gray A, Coussens L, Liao YC, Tsubokawa M: Human insulin receptor and its relationship to the tyrosine kinase family of oncogenes. Nature 1985; 313:756-761.

7 Ullrich A, Gray A, Tam AW, Yang-Feng T, Tsubokawa M, Collins C, Hezel W, Le Bon T, Kathuria S, Chen E: Insulin-like growth factor I receptor primary structure:

comparison with insulin receptor suggests structural determinants that define functional specificity. EMBO J 1986; 5: 2503-2512.
8 Yarden Y, Escobedo JA, Kuang WJ, Yang-Feng TL, Daniel TO, Tremble PM, Chen EY, Ando ME, Harkins RN, Francke U: Structure of the receptor for platelet-derived growth factor helps define a family of closely related growth factor receptors. Nature 1986; 323:226-232.
9 Yarden Y, Kuang WJ, Yang-Feng T, Coussens L, Munemitsu S, Dull TJ, Schlessinger J, Francke U, Ullrich A: Human proto-oncogene c-kit: A new cell surface receptor tyrosine kinase for an unidentified ligand. EMBO J 1987; 6:3341-3351.
10 Kawamoto T, Sato JD, Le A, Polikoff J, Sato GH, Mendelsohn J: Growth stimulation of A431 cells by epidermal growth factor: identification of high-affinity receptors for epidermal growth factor by an anti-receptor monoclonal antibody. Proc Natl Acad Sci USA 1983: 80;1337-1341.
11 Yamamoto T, Nishida T, Miyajima N, Kawai S, Ooi T, Toyoshima K: The erbB gene of avian erythroblastosis virus is a member of the src gene family. Cell 1983; 35:71-78.
12 Salomon DS, Brandt R, Ciardiello F, Normanno N: Epidermal growth factor-related peptides and their receptors in human malignancies. Crit Rev Oncol Hematol 1995; 19:183-232.
13 Wells A, EGF receptor. Int J Biochem Cell Biol 1999; 31:637-643.
14 Woodburn JR: The epidermal growth factor receptor and its inhibition in cancer therapy. Pharmacol Ther 1999; 82:241-250.
15 Slamon DJ, Clark GM, Wong SG, Levin WJ, Ullrich A, McGuire WL: Human breast cancer: correlation of relapse and survival with amplification of the HER-2/neu oncogene. Science 1987; 235:177-182.
16 Slamon DJ, Godolphin W, Jones LA, Holt JA, Wong SG, Keith DE, Levin WJ, Stuart SG, Udove J, Ullrich

A, Press MF: Studies of the Her-2/neu proto-oncogene in human breast and ovarian cancer. Science 1989; 244:707-712.

17 Gusterson BA, Gelber RD, Goldhirsch A, Price KN, Save-Soderborgh J, Anbazhagan R, Styles J, Rudenstam CM, Golout R, Reed R, Martinez-Tello F, Tiltman A, Torhorst J, Grigolato P, Bettelheim R, Neville AM, Bürki K, Castiglione M, Collins J, Lindtner J, Senn H-J: Prognostic Importnce of c-erbB-2 expression in breast cancer. International (Ludwig) Breast Cancer Study Group. J Clin Oncol 1992; 20:1049-1056.

18 Hynes NE, Stern DF: The biology of erbB-2/neu/HER-2 and its role in cancer. Biochim Biophys Acta 1994; 30:2-3.

19 Hudziak RM, Lewis GD, Winget M, Fendly BM, Shepard HM, Ullrich A: p185HER2 monoclonal antibody has antiproliferative effects in vitro and sensitizes human breast tumor cells to tumor necrosis factor. Mol Cell Biol 1989; 9:1165-1172.

20 Fendly BM, Winget M, Hudziak RM, Lipari MT, Napier MA, Ullrich A: Characterization of murine monoclonal antibodies reactive to either the human epidermal growth factor receptor HER2/neu gene product. Cancer Res 1990; 50:1550-1558.

21 Shepard HM, Lewis GD, Sarup JC, Fendly BM, Maneval D, Mordenti J, Figari I, Kotts CE, Palladino MA Jr, Ullrich A: Monoclonal antibody therapy of human cancer: taking the HER2 protooncogene to the clinic. J Clin Immunol 1991; 11:117-127.

22 Lewis GD, Figari I, Fendly B, Wong WL, Carter P, Gorman C, Shapard HM: Differential responses of human tumor cell lines to anti-p185HER2 monoclonal antibodies. Cancer Immunol Immunother 1993; 37:255-263.

23 Carter P, Presta L, Gorman CM, Ridgway JB, Henner D, Wong WL, Rowland AM, Kotts C, Carver ME, Shepard HM: Humanization of an anti-p185HER2

antibody for human cancer therapy. Proc Natl Acad Sci USA 1992; 89:4285-4289.
24 Anklesaria P: Gene therapy: a molecular approach to cancer treatment. Curr Opin Mol Ther 2000; 2:426-432.
25 Wagner RW: Gene inhibition using antisense oligodeoxynucleotides. Nature 1994; 372:333-335.
26 Wright L, Kearny P: Current status of ribozymes as gene therapy agents for cancer. Cancer Invest 2001; 19:495-509.

Figure and Table Titles and Legends

Figure 1
Translating Research into Cancer Therapies

Figure 2
Patient-specific Cancer Therapy

Table 1
Receptor Tyrosine Kinases: Targets for Therapeutic Intervention

EGFR = Epidermal growth factor receptor, CSF-1R = macrophage colony-stimulating factor-1 receptor, PDGFR = platelet-derived growth factor receptor, IGF-1R = insulin-like growth factor-1 receptor, SCFR = stem cell factor receptor.

Table 2
Target-specific Agents and Approaches for Anticancer Therapy

PDGFR = platelet-derived growth factor receptor, VEGF = vascular endothelial growth factor.

Table 1.

Receptor tyrosine kinase	Clinical indication or disease association
EGFR	Mammary carcinoma
	Squamous carcinoma
	Glioblastoma
	Bladder carcinoma
	Cervix carcinoma
	Non-small cell lung cancer
	Pancreatic carcinoma
HER2/*neu*	Mammary carcinoma
	Ovarian carcinoma
	Gastric carcinoma
	Pancreatic carcinoma
	Non-small cell lung cancer
c-fms/CSF-1R	Reproductive tract cancer
	Monocyte/macrophage/osteoclast
PDGFR□	Astrocytoma
	Restenosis
	Atherosclerosis
IGF-1R	Various cancers (anti-apoptosis)
c -kit/SCFR	gastro-intenstinal stromal tumor

Table 2.

General target	Specific target	Agent or approach
Signal transduction	***Growth factor receptors***	
	HER1 (EGFR)	Tarceva™, Iressa™
	HER2	Herceptin®
	Bcr-Abl	Glivec™
	Ras	Farnesyl transferase inhibitors
	Raf	Antisense oligonucleotides
Angiogenesis and metastasis	VEGFR2	SU5416
	VEGF	rhuMAb (Avastin™)
	Matrix metalloproteinases	Neovastat™
	Integrins	Vitaxin™
Tumour suppressor gene	p53	Gene therapy
	p16	Gene therapy
Cell cycle control	Cyclin-dependent kinases	Flavopiridol

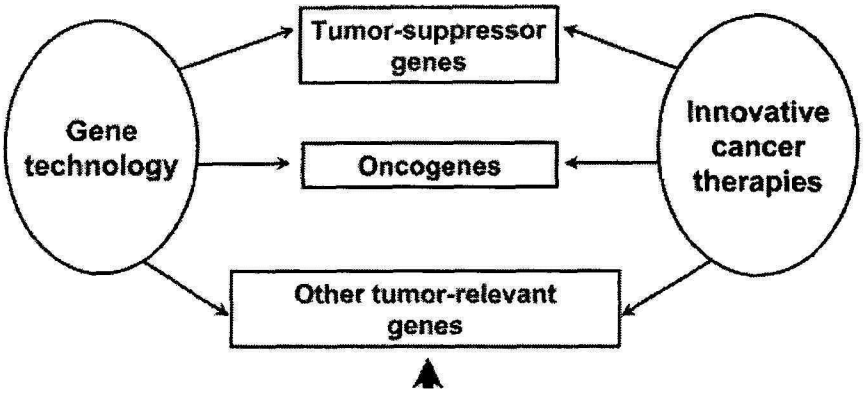
Tumor-suppressor genes
Gene technology
Oncogenes
Innovative cancer therapies
Other tumor-relevant genes

Fig. 1

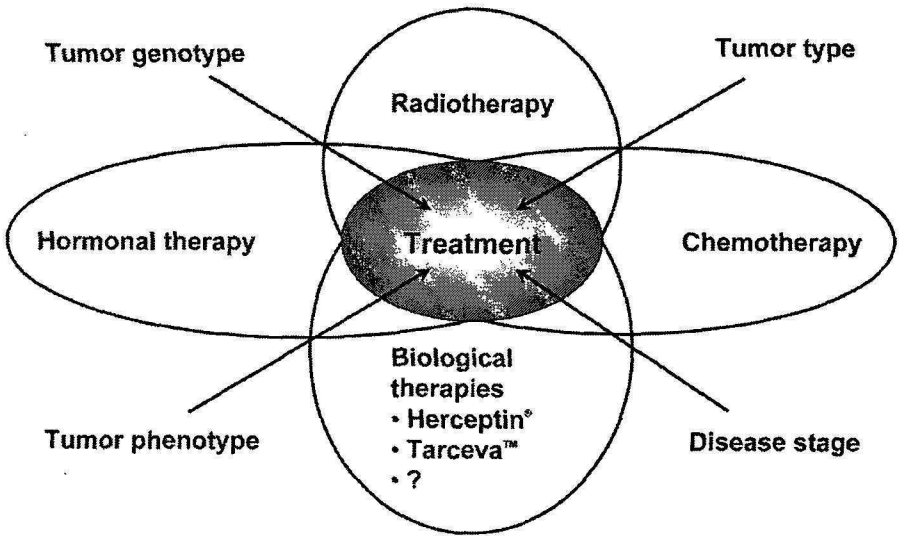
Tumor genotype
Tumor type
Radiotherapy
Hormonal therapy
Treatment
Chemotherapy
Biological therapies
• Herceptin®
• Tarceva™
• ?
Tumor phenotype
Disease stage

Fig. 2

WINNER OF THE 2003
KING FAISAL INTERNATIONAL PRIZE
FOR SCIENCE

Medal: King Faisal International Prize for Science

PROFESSOR M. FREDERICK HAWTHORNE

Co-Winner of the 2003 King Faisal International Prize for Science

Professor M. Frederick Hawthorne receives his prize from HRH Prince Sultan Ibn Abd Al-Aziz, *Second Deputy Primier, Minister of Defence and Aviation and Inspector General*

Synopsis of Achievements

Professor Hawthorne's research has been 'instrumental in the establishment of the field of carborane chemistry. He conceived and carried out the fusion of transition metals with carborane clusters, which led to the discovery of the huge fields of metallacarborane and metallaborane chemistry that extend throughout the periodic table. He sought and found homogeneous metallacarborane catalysts and new organometallic reactions characteristic of borane clusters. He also produced boron-labeled peptides, liposomes and algometric phosphate esters for use as boron-10 target compounds in boron neutron capture therapy of cancer. Most recently carboranes and polyhedral boranes are being developed as molecular manifolds for drug delivery, as pharmacophores groups in drug design and as components of molecular electronic devices and nanomachines. These and other accomplishments of Professor Hawthorne have prepared the promising future of polyhedral borane chemistry in science and technology.

Some Biomedical Applications of Aromatic Polyhedral Boranes and Their Progeny

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Abstract

The aromatic polyhedral boranes, and the vast array of structurally similar isoelectronic analogs formed by substitution of boron by other elements, have evolved into a major field of chemistry which provides useful applications available nowhere else in the periodic table. This brief paper describes established results and ongoing research in which aromatic polyhedral borane chemistry strongly assists biomedicine and pharmacology.

1. Introduction

The element boron neighbors carbon in the periodic table, exists in plentiful supply and reaches a long chemical arm across the periodic table to form stable compounds with many elements in diverse regions of the periodic table. However, the most important chemical property shared by boron and carbon is their ability to form huge families of discrete structures by bonding to themselves (so called "catenation"). Thus, boron atoms form stable bonds to other boron atoms when forming polyhedral borane cage structures. Carbon utilizes this catenation property when forming the carbon-carbon bonds required by organic chemistry. Advances made during the past fifty years have now established polyhedral borane species as the bases of a nearly infinite number of possible new structures containing elements from throughout the periodic table. The special relationship of boron and carbon (they differ by only

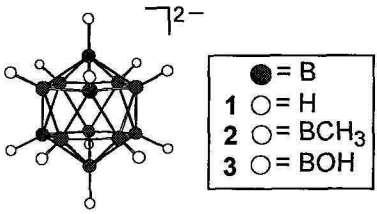
one nuclear proton and one valence electron) provides the basis of their facile interchange as seen in molecular structures exemplified by the existence of the polyhedral carboranes explained below. This new science supports the ever-increasing scope of molecular structures having extraordinary chemical, biological, thermal and photochemical stabilities. Such properties coupled with viable methods for the synthesis of these species provide unique applications not possible with other elements including carbon. Borane and hydrocarbon derivatives share many related structural and functional features. However, aside from essential chemical reactions, these species differ due to the fact that boranes have thus far been shown to be unaffected by the enzymes which have supported the evolution of life on this planet. Another unique feature of the boron component of boranes is its natural isotopic distribution of ^{10}B (20%) and ^{11}B (80%) accompanied by the very great propensity of a ^{10}B nucleus to capture a slow neutron and fission to produce ^{4}He and ^{7}Li nuclei (the boron neutron capture reaction). This process is accompanied by the liberation of about 2.4 MeV of kinetic energy, distributed among an alpha particle or $^{4}He^{2+}$, a ^{7}Li nucleus and an 0.5 MeV γ-photon.[1] As will be shown, this reaction is selective and useful in medicine since the neutron capture cross-section of the ^{10}B nucleus is $10^3 - 10^5$ larger than that of the light elements important in biological systems.[2]

2. Polyhedral Boranes as Structural Modules

The subject of this paper is the use of aromatic polyhedral borane and isoelectronic carborane species[3] as modules in the construction of a variety of species having biomedical functions and applications not possible when restricted to carbon-based chemistry.

The parent polyhedral borane, considered by many to be the benzene of the boranes, is the icosahedral [*closo*-$B_{12}H_{12}]^{2-}$,**1**, which has 26 electrons for cage-bonding.[4]

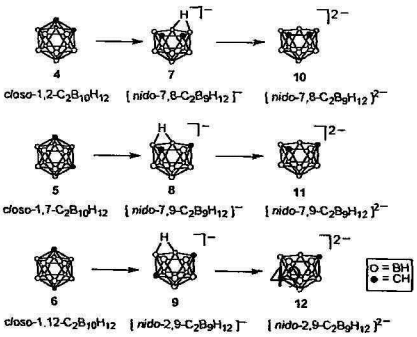
Twenty-four of these electrons are delocalized on the surface and two are



1, 2 and **3**

delocalized in the interior of the icosahedron.[5] The resulting stabilization of the icosahedral ion is such as to make this species extraordinarily stable and imbued with aromatic properties. Electrophilic substitution of the cage hydrogen atoms is a principal reaction giving, as an example, [*closo*-$B_{12}(CH_3)_{12}]^{2-}$[6], **2**, and [*closo*-$B_{12}(OH)_{12}]^{2-}$[7], **3**.

Substitution of two BH^- vertices by CH generates the isoelectronic, but uncharged, icosahedral carboranes, *closo*-$C_2B_{10}H_{12}$, which exists as the 1,2-, 1,7- and 1,12-isomers (**4**-**6**, respectively) depending upon the steric relationship of the two CH vertices to each other.[3] The carboranes are very stable thermally, but they undergo electrophilic substitution of hydrogen,[3] metallation at the CH vertices,[3] reversible two-electron redox reactions[3] and the very novel and important deboronation reaction in which a specific BH vertex (depending upon the carborane isomer) is removed with base generating an open pentagonal face having an extra acidic hydrogen in its



periphery. These are the isomeric [*nido*-7,8-,7,9- and 2,9-$C_2B_9H_{12}]^-$ ions (**7-9,** respectively).[8] These anions are very easily halogenated and converted by base to their corresponding [*nido*-7,8-, 7,9- and 2,9-$C_2B_9H_{11}]^{2-}$ dicarbollide ions[9] (**10-12**, respectively) having six
electrons delocalized on the pentagonal face. The latter species are isolobal and isoelectronic with the cyclopentadienide ion, $[\eta^5\text{-}C_5H_5]^-$, employed in the syntheses of metallocenes such as ferrocene $(\eta^5\text{-}C_5H_5)_2Fe$. Consequently, transition metal metallacarboranes (Figure 1) were discovered thereby opening a new field of chemistry[10]

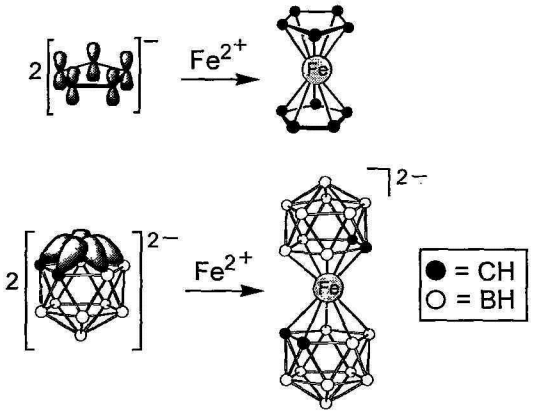


Figure 1. Comparison of biscyclopentadienyl iron(II) with dicarbollyl iron(II) showing similar π-bonding modes.

which bridged the existing fields of transition metal coordination chemistry and polyhedral boranes. This new field rapidly prospered, metallacarboranes from throughout the periodic table have been investigated and many novel applications found.

The aromatic [*closo*-$B_{10}H_{10}]^{2-}$ ion[11], **13**, has the geometry of a bicappd square antiprism and it resembles icosahedral [*closo*-$B_{12}H_{12}]^{2-}$ in its chemical reactions although it is somewhat more reactive than the latter.

Oxidation of [*closo*-$B_{10}H_{10}$]$^{2-}$ with a variety of chemical oxidants affords a unique product ion, [*trans*-$B_{20}H_{18}$]$^{2-}$. This anion contains two [*closo*-$B_{10}H_9$]$^-$ fragments joined by a pair of B-B-B 3-center bonds.[12] Two-electron reduction of this novel ion produces [$B_{20}H_{18}$]$^{4-}$ as three interconvertible isomers with formal [*closo*-$B_{10}H_9$]$^{2-}$ radical fragments joined by a B-B sigma bond in three possible geometric arrangements, e^2, ae and a^2 where e = an equatorial linkage site and a = an apical linkage site. The e^2 isomer is least stable and the a^2 isomer most stable. Equilibration is accomplished using acid catalysis and isomeric [$B_{20}H_{19}$]$^{3-}$ intermediates.[13] The chemistry of B_{20} derivatives is presented in Figure 2.

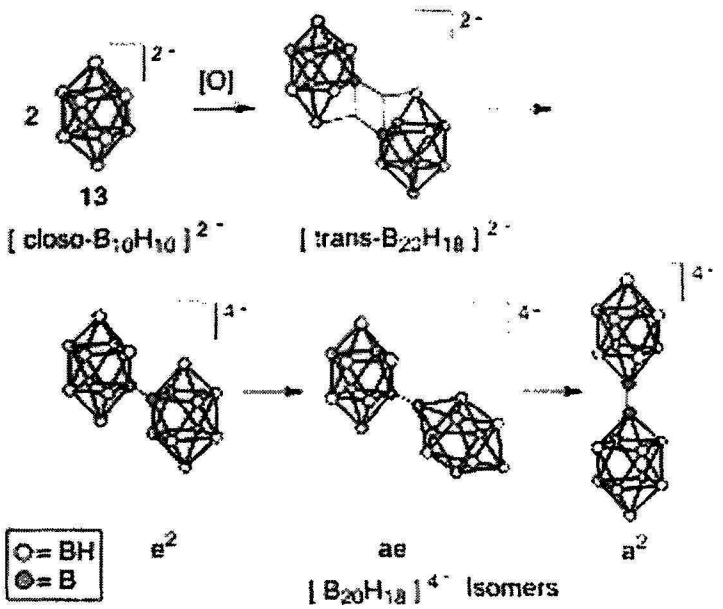


Figure 2. Structure of [*closo*-$B_{10}H_{10}$]$^{2-}$, **13**, its oxidation to [*trans*-$B_{20}H_{18}$]$^{2-}$ and subsequent reduction to the three [*closo*-$B_{20}H_{18}$]$^{4-}$ isomers.

With these modules in hand, we now proceed to their utilization in biomedical applications.

3. Boron Neutron Capture Therapy of Cancer (BNCT)

The propensity of the ^{10}B nucleus to capture a slow neutron and rapidly fission to ^{4}He and ^{7}Li nuclei is unique among the elements found in living systems as described above.[1] Consequently, a binary radiation therapy modality has been devised[2] in which a ^{10}B-containing molecule is selectively prepositioned in, or near, a tumor cell nucleus and the tagged malignant cell then irradiated with slow (thermal) neutrons.

Thermal neutrons are themselves innocuous to living cells. However, neutron capture by the ^{10}B-label in a malignant cell results in nuclear fission causing DNA damage in the targeted cell and a low probability of collateral damage to neighboring cells due to the short trajectories of the heavy ion fission products. This method is even more attractive since the therapeutic process may be terminated at any moment by removing the neutron source (usually a beam originating in a nuclear reactor). The chemical research required to develop BNCT involves the design and synthesis of relatively nontoxic boron-rich compounds and the complementary tumor cell-selective delivery methods needed to reach only malignant cells and in quantities of about 10^{-3}-10^{-4} M within the tumor cell.[14,15] While boron compounds are plentiful, few are known which can provide high boron content coupled to low toxicity and tumor cell-selective delivery at high concentrations (normal pharmaceuticals function at 10^{-6}-10^{-9} M concentrations in cells). Thus, one strives to design boron-rich agents which carry many B-atoms per targeted cell receptor and combine these properties with the ability to enter the nucleus of targeted cells once the compound arrives in the cytoplasm.[14,15] Two such systems are (1) unilamellar cell-targeting liposomes encapsulating an aqueous solution of a suitable water-soluble boron compound[16] and (2) a new type of relatively nontoxic boron-rich oligomeric phosphate diesters which rapidly enter cell nuclei and are retained for

many hours.[17] Liposomes (Figure 3) are 30-100 nm in diameter and with a bilayer constructed from conventional

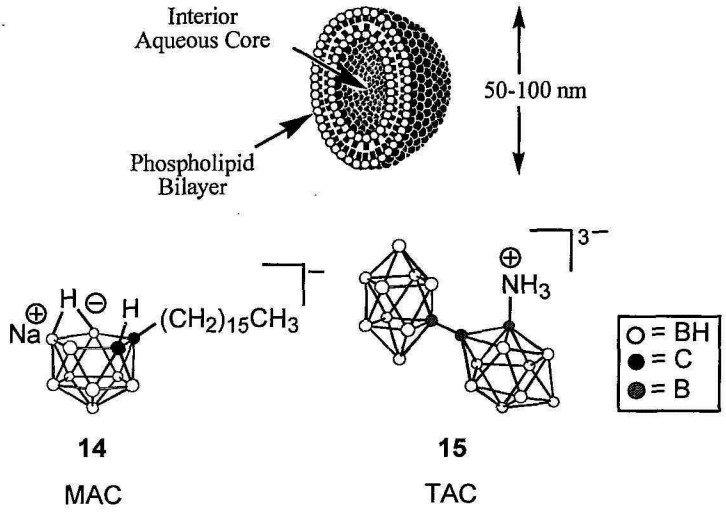


Figure 3. Depiction of a typical unilamellar liposome; MAC, 14, and TAC, 15, constituents of the lipid bilayer and aqueous core respectively.

distearoylphosphocholine/cholesterol (1:1 molar) containing variable quantities of the lipidic Na[*nido*-7-*n*-$C_{16}H_{33}$-7,8-$C_2B_9H_{11}$], MAC, **14**, to enhance boron concentration in the liposome.[18] The internal aqueous phase was hypertonic with respect to blood serum and contained experimental Na_3[1-(2`-$B_{10}H_9$)-2-$NH_3B_{10}H_8$], TAC, **15**. Injected (i.v.) doses of 15 mg B/Kg body weight with balb/C mice bearing EMT6 adenocarcinoma xenografts are easily tolerated. Time course biodistribution data prove that tumor tissue selectively accretes boron (> 35µg B/g tissue or 10^9 B atoms per cell) in the therapeutic range reaching a maximum after approximately 30 hours and slowly decreasing with time. A series of *in vitro* experiments using fluorescence microscopy and fluorophore-labeled lisposome components have demonstrated cellular uptake of typical liposomes specifically labeled in the aqueous core (TAC) and in the bilayer (MAC).[19] Liposomes of this type could be labeled with

ligands (antibodies) for tumor cell receptors however, the high selectivity of boron for tumor (5:1 to 20:1 depending on tissue) shown with these small liposomes makes this unnecessary. The high tumor selectivity observed must arise from preferential leakage of small liposomes through immature vasculature only present in the rapidly growing tumor. Free liposomes which escape the vasculature may then contact tumor cells and be taken up by any of a large number of possible processes. Experimental correlation of the structure/activity of the most effective compounds for boron uptake has shown ligand-substituted $[B_{20}H_{18}]^{4-}$ ions, $[B_{20}H_{17}L]^{3-}$(L: such as NH_3 replaces $H:^-$), to be most effective. This is thought to be due to intracellular 2-electron oxidation of the latter species to a very electrophilic and reactive $[B_{20}H_{17}L]^-$ which immediately reacts with amino or other nucleophilic groups available in the cytoplasm protein[16] (Figure 4). This would effectively anchor the B-delivery vehicle near the cell nucleus. The attractive biodistribution data obtained, the flexibility of liposome syntheses and the many modifications possible in therapeutic procedures make liposomes strong candidates for future evaluation leading to human use.

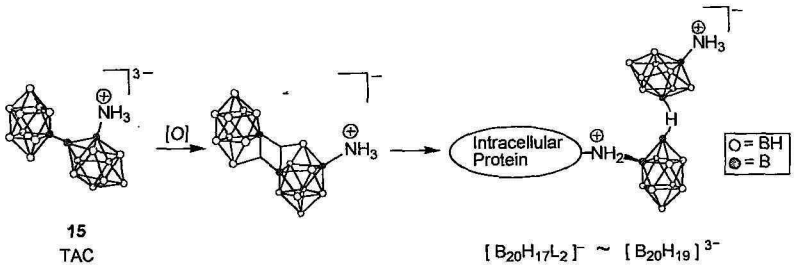
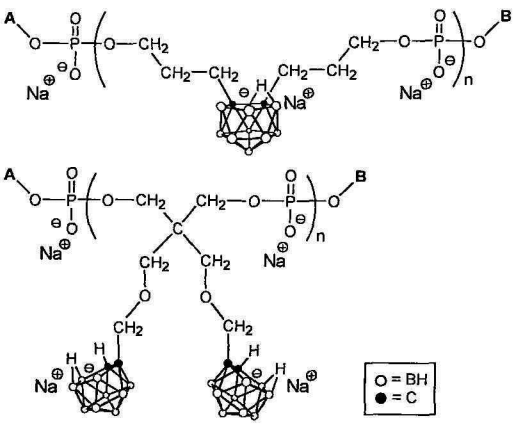


Figure 4. Possible reactions resulting in covalent binding of B_{20} species to intracellular protein following *in situ* oxidation of $[B_{20}H_{17}NH_3]^{3-}$ to electrophilic $[B_{20}H_{17}NH_3]^-$.

Oligomeric phosphate diesters (OPDs) derived from a library of existing diols which contain bivalent and hydrophilic

[*nido*-7,8-$C_2B_9H_{11}^-$]$^-$ modules have been synthesized from phosphoramidite precursors using the same methodology employed for DNA syntheses.[20] This provides fixed chain length, controlled sequence of modular diols and the ability to incorporate a variety of functional modules and labels in the resulting oligomer at predetermined points (Figure 5). The high boron content of OPDs coupled with their low toxicities and their functionalization for conjugation to tumor-associated bioligand molecules makes them attractive for that purpose. Surprisingly, the results of single-cell microinjection of large quantities of fluorescence-labeled OPDs in green monkey kidney cells proved that once in the cytoplasm OPDs rapidly translocate to the cell nucleus and remain there for many hours without apparent damage to the cell.[21] Up to 10^9 B-atoms (as OPD) have been employed in single cells. This is an ample therapeutic dose for BNCT. The translocation process to the nucleus is most likely



A = Thymidine-, B = -$(CH_2)_6NH_2$, n = 10
A = Biotin-C(O)NH$(CH_2)_6$-, B = -$(CH_2)_6NH_2$, n = 20
A = Thymidine-, B = -CH_3CH(Fluorescein)$CH_2OP(O)_2O(CH_2)_6NH_2$, n = 20
A = Thymidine-$OP(O)_2O$-CH_2CH(Fluorescein)CH_2-, B = -$(CH_2)_6SH$, n = 20

Figure 5. Representative oligomeric phosphate diesters (OPDs) equipped with typical substituent groups.

diffusion driven while the tight binding of the OPD to nuclear components remains uncharacterized. However, it may be possible for the highly charged (dinegative repeating units) OPD to displace DNA from its cationic anchoring sites without damage to the DNA. Aside from BNCT, drug or nucleotide delivery may be possible using conjugated OPD moieties as translocation agents. The use of OPDs for boron-targeting of nuclear DNA is very attractive. The remaining obstacle to success is the need to attach OPDs and their ancillary groups to specific cell-targeting bioligands and provide plasma membrane penetration, as well. This may be possible using liposomes as transport vehicles for OPDs or for OPD conjugates with drugs or nucleotides.

4. Radioimaging and Radiotherapy

Contemporary uses of radionuclides in radioimaging and cell-targeted cancer therapy involve the complexation of radiometals to organic aminocarboxylates or attachment of radioiodine isotopes to activated aryl groups such as tyrosine residues by electrophilic substitution. Each of these modes of attachment is subject to later release of the free radionuclide by enzymatic degradation of the organic carrier moiety (catabolism). This potentially dangerous *in vivo* release of free radionuclide would be minimized if catabolism could be prevented by bonding the necessary radiometals to enzymatically stable as well as inorganic metal chelation agents and radioiodine to kinetically stable inorganic substrates. The scope of aromatic borane chemistry provides logical choices for this approach; radiometals could be held tightly in aromatic metallacarborane structures while aromatic polyhedral borane anions were known to rapidly undergo electrophilic substitution with iodine to form robust B-I bonds not subject to the enzymatic attack affecting C-I bonds in organic carriers. These borane and carborane radiocarriers would be functionalized and linked to biomolecules such as antibodies capable of selective binding to particular cell types. This methodology could be applied to radioimaging or radiotherapy depending upon the identity of the radionuclide selected and its characteristic radiation.

The iodination of the [*nido*-7,8-$C_2B_9H_{12}$]$^-$ ion with elemental iodine proceeds at a rapid rate to form [*nido*-9-I-7,8-$C_2B_9H_{11}$]$^-$ with the iodine substituted on the periphery of the open face of the dicarbollide ion.[22] Later extension of this work (Figure 6) using

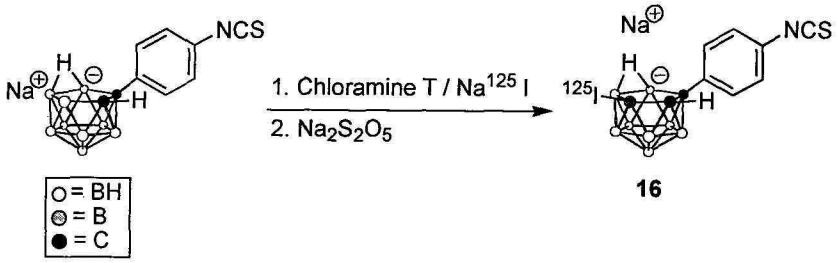


Figure 6. Radioiodination of functionalized *nido*-carborane anion to be followed by its conjugation to a biomolecule.

[*nido*-7-(*p*-C_6H_4NCS)-7,8- $C_2B_9H_{11}$]$^-$ with I^- and chloramine –T as the iodination reagent yielded [*nido*-7-(*p*-C_6H_4NCS)-9-I-7,8-$C_2B_9H_{10}$]$^-$ [23], **16**. Since any of the radioisotopes of iodine could be used in this procedure, the iodinated isothiocyanate derivative could be conjugated with biomolecules such as antibodies by reaction with free -NH_2 groups. The resulting conjugate could then be employed for γ-imaging or β-therapy of cancer. Later work has suggested the facile radioiodination of the [*closo*-B_nH_n]$^{2-}$ (n = 10 and 12) modules following suitable functionalization for conjugation to cell-specific bioligands by cage amination or hydroxylation and subsequent linker attachment.[24]

The incorporation of transition radiometals in metallacarborane sandwich structures carrying linker groups for biomolecule conjugation has been demonstrated through the synthesis of so-called "Venus Flytrap" (VF) Complexes.[25] These species display very strong π-bonding of the transition metal to a pair of enzyme-stable [*nido*-7,9-

$C_2B_9H_{11}]^{2-}$ ligands which, in turn, are linked to a functional group for biomolecule conjugation (Figure 7). The syntheses of many VF complexes may be conducted in

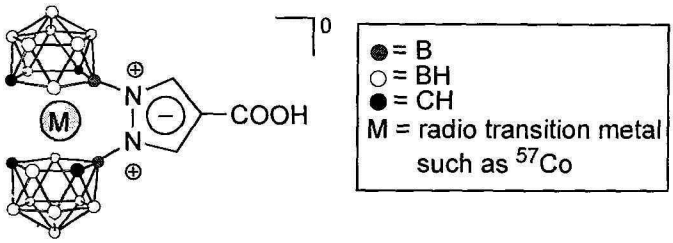


Figure 7. A Venus Flytrap complex containing a radioactive transition metal and a carboxyl group for biomolecule conjugation.

aqueous media, thus expediting the production of the time-sensitive radiolabeled product. Radioimaging[26] of tumor-bearing (LS174T murine adenocarcinoma) mice was accomplished using $^{57}Co(t_{1/2}271d,\gamma)$ in the depicted ^{57}CoVF conjugated to T84.66 monoclonal antibody (anti-carcinoembryonic antigen IgGl). Excellent imaging data were obtained and ^{57}Co was shown to be excreted by the biliary route as the VF complex. This use of cluster bonding to sequester radiotransition metals within aromatic carborane or polyhedral borane structures could, in principle, be extended to other transition metals such as ^{67}Cu, ^{99m}Tc, ^{105}Rh, ^{186}Re and others. Those complexes having formal d^6 metal centers are most stable since the 18-electron rule is obeyed in VF structures (each 7,9-dicarbollide ligand contributes 6-electrons for binding). Most recently, new developments in Tc carbonyl chemistry has allowed the synthesis of ^{99m}Tc and ^{186}Re radiopharmaceuticals [27], **17**, (Figure 8) based upon this scheme and analogous to the

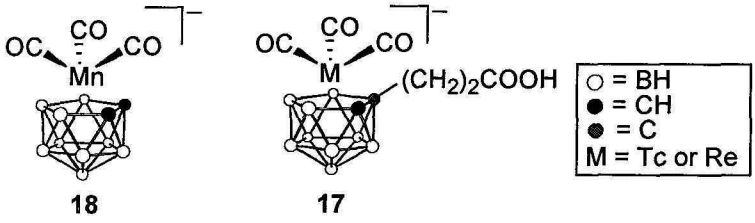


Figure 8. Radiopharmaceuticals (ref. 27, 28) based upon radiometal carbonyl derivative of dicarbollide ligand (M = Tc or Re).

previously known manganese tricarbonyl derivative [*closo*-3,3,3-$(CO)_3$-3,1,2-$MnC_2B_9H_{11}]^-$ [28], **18**. As before, functionalized linker chains are attached to achieve biomolecule conjugation.

Advances in radiopharmacy have been possible through their use of polyhedral borane and carborane carrier modules. This trend will undoubtedly continue and eventually involve the use of α-particle emitters such as ^{212}Bi and ^{211}At in cell-targeted cancer therapy giving results similar to those of BNCT without the binary feature which controls treatment duration.

5. Hydrophobic Modules for Pharmaceutical Design

The recently acquired ability of chemical biologists to ascertain the size, shape and polarity of active binding sites present in enzymes and other important types of biomolecules has vastly improved the pharmacologist's ability to tailor the syntheses of specific active site inhibitors as effective pharmaceuticals. Computational chemistry combined with structural information available from X-ray diffraction and high resolution NMR provide the basis of contemporary pharmaceutical design. One type of structural module often in demand, but not clearly defined, is a large, spherical and hydrophobic group which could be used to enhance inhibition of enzymes by filling one or more hydrophobic pockets in the enzyme active site.[29] This

module might be described as a near spherical hydrophobic group with a van der Waals diameter of about 10Å. Adamantane and C_{60} are candidates from the past with limited size in the former case and limited chemical flexibility coupled to a hard carbon surface in the latter. Consequently, the need for such a hydrophobic module exists which may be satisfied with nearly spherical and highly methylated derivatives of nonpolar and extraordinarily stable *closo*-1,12-$C_2B_{10}H_{12}$, **5**: *closo*-1,12-$C_2B_{10}(CH_3)_{12}$, or *closo*-1,12-$(H)_2$-1,12- $C_2B_{10}(CH_3)_{10}$. The latter species, synthesized by electrophilic methylation of BH vertices, have van der Waal diameters of about 10Å and they may be derivatized for incorporation within organic molecules at cage C-H vertices or at a B-CH_3 group using the photochemical Barton reaction (Figure 9) forming an aldoxine function by C-H bond

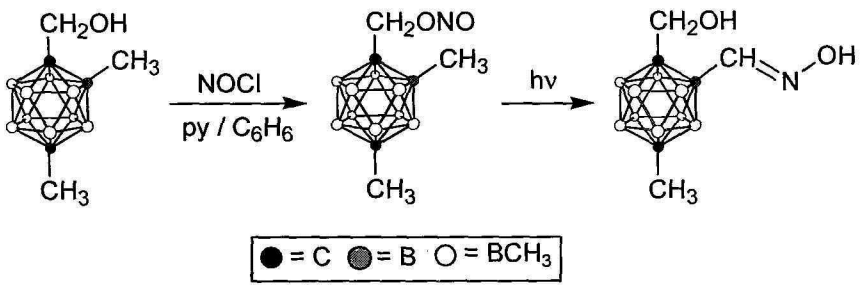


Figure 9. The Barton reaction developed for steroid chemistry applied to suitably substituted *p*-carborane derivatives.

activation. Research is in progress which employs the B-permethylated carborane cage to fill a hydrophobic pocket present in the thrombin enzyme active site. The thrombin inhibitor was designed by computational methods coupled to X-ray diffraction studies. The results of this and related research will be reported elsewhere. Success in this venture would encourage similar studies with additional enzyme systems and the use of bulky carboranes as hydrophobes in general pharmaceutical design.

6. Delivery Vehicles with Twelvefold Functionality: Closomers

Recent studies have led to the facile synthesis of the [*closo*-$B_{12}(OH)_{12}]^{2-}$ ion[7], **3**, by hydroxylation of [*closo*-$B_{12}H_{12}]^{2-}$ using hydrogen peroxide. In itself, this is a remarkable discovery since the [*closo*-$B_{12}(OH)_{12}]^{2-}$ ion retains its 26-electron set of core electrons as in its precursor [*closo*-$B_{12}H_{12}]^{2-}$. All twelve hydroxyl groups present on the icosahedral surface display chemical reactivity reminiscent of simple alcohols. Thus, carboxylate ester, carbamate ester and alkyl ether formation take place under normal conditions and twelvefold redundancy is the rule.[30,31] Linkages of this type can be employed to anchor bifunctional linkers whose termini may be used for conjugation to a wide variety of useful species ranging from chemotherapeutic agents to radiopharmaceuticals for imaging or therapy to magnetic resonance imaging contrast agents and boron-rich polyhedral species for BNCT (Figure 10). Since the icosahedral core of these species differs from that of the better known dendrimers, the borane species have been designated[30] as "closomers".

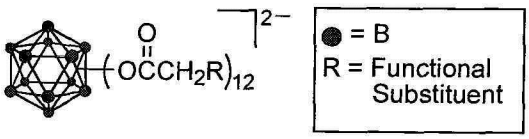


Figure 10. Typical closomer ester bearing twelve copies of a useful functional substituent.

Methods are in hand for the chemical differentiation of one or more regiospecific BOH vertices for use as attachment points for linkers terminating in specific cell-targeting bioligands. Studies are under way which will allow the conjugation of the remaining BOH vertices to linkers having orthogonal chemistry for the purpose of attaching species which provide a desired function such as those functions mentioned above (Figure 11). In this way many

copies of a desired moiety may be targeted to specific cells for a variety of purposes. Results of these studies will be reported elsewhere as they develop.

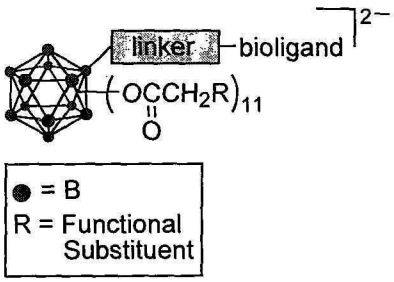


Figure 11. A closomer ester with eleven copies of a useful functional substituent and a single copy of a functionalized linker group for conjugation to a cell-targeting biomolecule.

7. The Future

The applications of aromatic polyhedral borane and carborane chemistry to just a portion of one field of endeavor, biomedicine, is briefly described here. Other fields of science and technology could provide the bases of similar surveys of completed and ongoing research. These alternative fields would be new materials and catalysts, supramolecular chemistry, separations science, molecular electronics, nanotechnology and others. The future of modern borane chemistry awaits its fulfillment and astounding results are assured.

8. Acknowledgements

The author thanks his coworkers past and present for their contributions and loyal support, the National Science Foundation, the Department of Energy and the National Institutes of Health for generous financial assistance and the King Faisal Foundation for the honor of presenting this work in conjunction with the 2003 King Faisal International Prize in Science.

Selected References:

1. P.G. Kruger, *Proc. Natl. Acad. Sci. USA, 26*, 181, **1940**.
2. G.L. Locher, *Am. J. Reoentgenology, 36*, 1, **1936**.
3. R.N. Grimes, *Carboranes*, Academic Press, New York, **1970**.
4. M.F. Hawthorne and A.R. Pitochelli, *J. Am. Chem. Soc., 82,* 3228, **1960**.
5. R. Hoffman and W.N. Lipscomb, *J. Chem. Phys., 36,* 2179, **1962**.
6. T. Peymann, C.B. Knobler, S. I. Khan and M.F. Hawthorne, *Inorg. Chem., 40*, 1291, **2001**.
7. T. Peymann, C.B. Knobler, S.I. Khan and M.F. Hawthorne, *J. Am. Chem. Soc., 123*, 2182, **2001**.
8. M.F. Hawthorne, D.C. Young, P.M. Garrett, D.A. Owen, S.G. Schwerin, F.N. Tebbe and P.A. Wegner, *J. Am. Chem. Soc., 90*, 862, **1968**.
9. M.F. Hawthorne, D.C. Young and P.A. Wegner, *J. Am. Chem. Soc., 87*, 1818, **1965**.
10. M.F. Hawthorne, *Endeavor, 25*, 145, **1966**.
11. W.N. Lipscomb, A.R. Pitochelli and M.F. Hawthorne, *J. Am. Chem. Soc., 81*, 5833, **1959**.
12. R.L. Pilling, F.N. Tebbe, M.F. Hawthorne and E.A. Pier, *Proc. Chem. Soc. (London), 402*, **1964**.
13. M.F. Hawthorne, R.L. Pilling and P.F. Stokely, *J. Am. Chem. Soc., 87*, 1893, **1965**.
14. M.F. Hawthorne, *Angew. Chem. Int. Ed. (English), 32*, 950, **1993**.
15. A.H. Soloway, W. Tjarks, B.A. Barnum, F.-G. Rang, R.F. Barth, I.M. Codogni and J.G. Wilson, *Chem. Rev., 1515*, **1998**.
16. D.A. Feakes, K. Shelly, C.B. Knobler and M.F. Hawthorne, *Proc. Natl. Acad. Sci. USA, 91*, 3029, **1994**.
17. K. Drechsel, C.S. Lee, E.W. Leung, R.R. Kane and M.F. Hawthorne, *Tetrahedron Lett., 35*, 6217, **1994**.
18. D.A. Feakes, K. Shelly and M.F. Hawthorne, *Proc. Natl. Acad. Sci. USA, 92*, 1367, **1995**.
19. R.M. Straubinger, *Department of Energy Subcontract Report, Lisposome Biodynamics*, **1995**, Idaho National

Engineering and Environmental Laboratory, The Department of Energy.
20. R.R. Kane, K. Drechsel and M.F. Hawthorne, *J. Am. Chem. Soc., 115*, 8853, **1993**.
21. A. Nakanishi, L. Guan, R.R. Kane, H. Kasamatsu and M.F. Hawthorne, *Proc. Natl. Acad. Sci. USA, 96*, 238, **1999**.
22. F.P. Olsen and M.F. Hawthorne, *Inorg. Chem., 4*, 1839, **1965**.
23. E.A. Mizusawa, M.R. Thompson and M.F. Hawthorne, *Inorg. Chem., 24*, 1911, **1985**.
24. V.Talmachev and S. Sjöberg, *Coll. Czech. Chem. Com., 67*, 913, **2002**.
25. M.F. Hawthorne, A. Varadarajan, C.B. Knobler, S. Chakrobarti, R.J. Paxton, B.G. Beatty and F.L.Curtis, *J. Am. Chem. Soc., 112*, 5365, **1990**.
26. R.J. Paxton, B.G. Beatty, M.F. Hawthorne, A. Varadarajan, L.E. Williams, F.L. Curtis, C.B. Knobler, J.D. Beatty and J.E. Shively, *Proc. Natl. Acad. Sci. USA, 88*, 3387, **1991**.
27. J.F. Valliant, P. Morel, P. Schaffer and J.H. Kaldis, *Inorg. Chem., 41*, 628, **2002**.
28. M.F. Hawthorne and T.D. Andrews, *J. Am. Chem. Soc., 87*, 2496, **1965**.
29. M.F. Hawthorne, *Contemporary Borane Chemistry*. Edited by M.G. Davidson, A.K. Hughes, T.B. Marder and K. Wade, Special Publication 253 of the Royal Society of Chemistry, page 197, **2002**.
30. A. Maderna, C.B. Knobler and M.F. Hawthorne, *Angew. Chem., 40*, 1662, **2001**.
31. T. Peymann, C.B. Knobler, S.I. Kahn and M.F. Hawthorne, *Angew. Chem., 40*, 1664 **2001**.

PROFESSOR KOJI NAKANISHI

Co-Winner of the 2003 King Faisal International Prize for Science

Professor Koji Nakanishi receives his prize from HRH Prince Sultan Ibn Abd Al-Aziz, *Second Deputy Primier, Minister of Defence and Aviation and Inspector General*

Synopsis of Achievements

Professor Nakanishi is the world's leader in the isolation and structure determination of biologically active natural products. He designed versatile techniques to study natural products and bioactivity beyond the limits imposed by the miniscule quantity of material. This enabled him to determine the structure of more than 350 compounds, many of which are members of new classes and/or endogenously occurring. By applying atomic level structure determination techniques to the study of biopolymers, he was able to elucidate the structural basis for the activity of some carcinogenic substances, neurotoxins and anticancer agents. Of particular importance are his studies on the interaction of light with rhodopsin (the pigment molecule responsible for vision), in which numerous basic aspects have been resolved through preparation of more than 100 rhodopsin analogs. These studies are close to solving the mystery of macular degeneration, which affects all humans and may lead to blindness for which no treatment is presently known.

Lessons from Nature

Professor Koji Nakanishi

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New York, NY 10027, USA

Natural products is the oldest branch in organic chemistry. It started with a curiosity regarding biologically activity, folk medicinal cures, odor, fermentation, etc. In the early days of chemistry, natural products chemistry dealt with isolation and structure determinations, followed by synthetic and biosynthetic investigations. With the advancement in techniques the trend shifted towards assay-monitored isolation and mode of action, namely, understanding the ligand and receptor interactions.

Organic chemists, especially those involved in structural studies have the techniques and general knowledge as to how to tackle such projects but are not directly exposed to biological or physiological phenomena,. Nature is complex, and thus multidisciplinary collaborative research is essential. A description of. some studies we have performed in the general area of bioorganic studies of natural products follows.

8. Insect / crustacean molting hormones and crustacean molting inhibitor (Fig. 1).

Ecdysone **1**, the precursor of the universal molting hormone of insects and crustacens was isolated from male *Bombyx mori* (silkworm) by Butenand, Karlson, and coworkers (1965) and its structure was determined by X-ray (by Huber / Hoppe,) This was soon followed by characterization of the genuine molting hormone 20-hydroxyecdysone **2**. On the other hand, search for anticancer compounds from a Taiwanese plant *Podocarpus nakaii*, led to isolation and characterization of ponasterone A **3**, which only differed from **2** by the lack of a hydroxyl group at C-25. Insect molting assays of ponasterone A

showed that it was 10-fold more active than **2**. Furthermore, in contrast to the minuscule availability of **2**, the plant had yielded multigram quantities of ponasterone A and other similar compounds with high insect hormone activity (1966).[1] Takemoto also had isolated a similar compound inokosterone from a common Japanese weed.[2] Over 80 ecdysteroids have since been isolated from plants. The abundance and variations in structures and activities of these phytoecdysteroids had a huge impact on insect physiology.[3]

It was known that removal of eyestalks from crustaceans leads to precocious ecdysis, thus suggesting the presence of a "molt-inhibiting hormone (MIH)" in the eyestalk. Using 4,000 blue crabs *Callinectes sapidus* and years of frustrating isolation sstudies, Naya et al. finally clarified the picture of the crustacean molt inhibition.[4,5] The bioassay that depended on the production /inhibition of ecdysone by the Y organs located at the stem of the eye stalk, the site of ecdysone biosynthesis, did not work during a particular summer or even over periods longer than a year, whether the crabs were collected in the United States or in Japan; we attribute this to a world-wide cold summer. Naya finally succeeded in isolating 0.7 mg of the molt inhibitor and identified this endogenous compound contained in the X organ of the eyestalk as 3-hydroxy-*L*-kynurenine (3-OH-K), a key metabolite of L-tryptophan.[4]

Further during the flow of 3-OH-K to the Y-organ complex, it was found to converd into xanthurenic acid, the real ecdysone biosynthesis inhibitor. Xanthurenic acid interferes with the hydroxylation of cholestrol to ecdysone by interacting with the P-450 system.[5]

2. Ginkgolides (Fig. 2).

Although no special biological activity was found at the time of studies in 1964, later they were found to be potent antagonists of platelet activating factor (PAFR). The Ginkgo biloba extract is already mentioned in the Chinese Materia Medica 5,000 years ago. The annual sales of the crude extract

as a phytopharmaceutical and dietary supplement, reputed to improve memory and sharpen mental focus, was $ 1 billion in 1998.

It was during the course of structural elucidation of these complex pentacyclic compounds that the power of intramolecular nuclear Overhauser effect (NOE) in structural studies was first demonstrated[6] These ancient and extremely stable molecules displayed a series of unprecedented chemical reactions, which were interpreted, with the help of NOE, and led to the unique cage molecular structures consisting of six five-membered rings. Statistics show that the G. biloba extract helps enhance memory and has a positive effect in slowing Alzheimers disease. After a lapse of over 35 years, we haved resumed ginkgolide studies aiming to clarify the mode of binding of the ginkgolides to PAFR.[7]

Shark repellents (Fig. 3).

In 1978 we were told that there were soles similar to the shark-repelling Red Sea Moses sole around Okinawa. This led to the isolation and charactherization of the shark-repelleling pavonins / mosesins[8] and pardaxins (32 peptides).[9] As shown in Fig. 3 the six pavonins and four mosessins owe their toxicity to their detergent structures. The toxicity of the pardaxins also arises from their amphiphilic structure. The isolation was performed by monitoring the bleeding of killy fish. The isolation of shark repellents secreted presented an extremely difficult challenge that had hindered the efforts of many groups. Upon attack by a shark, the sole immediately secretes these detergents so that the shark makes a sharp U-turn and the sole is left unharmed.

3. Benzpyrene / DNA adduct (Fig. 4).

Polyaromatic hydrocarbons (PAH) exemplified by benzpyrene (BP) and dimethylbenzanthracene (DMBA) are potent carcinogens; the high incidence of cancer seen in chimneysweeps is attributed to the high concentration of PAH in

chimney soot. Until the first structures of the benzo[a]pyrene (BP) adducts were determined,[10] structures of polyaromatic hydrocarbon (PAH)/nucleic acid adducts were not known. BP/DNA adduct structures were also elucidated by independently by Koreeda and collaborators.[11] PAH taken into the body is normally oxidized and secreted without causing damage. However, as shown in Fig. 4 for BP, if it is oxidized to the depicted diolepoxide ("ultimate carcinogen"), this will react with DNA to yield adducts leading to cancer.

In these studies, the tritiated PAH is incubated with the tissue, the nucleic acid fractions are collected, hydrolyzed, and the pure adducts are isolated by monitoring tritiated HPLC peaks. As the quantity of these adducts are far too small for any proper structural studies, the suspected activated metabolites, such as the diol epoxides are chemically synthesized and reacted with various nucleosides or homopolynucleotides under various conditions until an HPLC peak with the same retention time is obtained. The isolated cold adduct, usually ca.1 mg, is submitted to structural studies. Difficulties lie in characterizing the active metabolite which gives the PAH/nucleic acid adducts. Identification of these ultimate carcinogen, e.g., the epoxide in Fig. 5, is extremely challenging. After characterization of the activated PAH metabolite, isolation and structure determination of the adducts also present a problem due to the minuscule amount of the adduct with many polar groups, and the multiple linking possibilities between PAH and nucleic acid moieties.

The nucleic acid moiety G lacks protons and hence NMR cannot be used to determine its linkage point to BP. Two micromethods were developed to serve this purpose.[12] One relied on the difference in pK values of guanines substituted at various positions. The intense UV of the PAH portion overlying the G prevents UV measurements; however, the split CD curve of the adduct is due to spatial interaction of the G and BP moieties, and therefore if the absorption changes with pH (in this case the G moiety) the split CD should also change with pH. A plot of the CD extrema against pH indicated pK' of 2.1 and 9.1, i.e., the BP adduct is 2-alkylsubstituted G. The second method

employed difference Fourier transform infrared spectroscopy (SEDIR), a technique that proved to be immensely powerful (see mitomycin, next section).

4. Mitomycin C / DNA crosslinked adduct (Fig. 5).

The structure of adducts formed between mitomycin C (MC) and DNA, including the elusive cross-linked adduct shown in Fig. 5,[13] clarified the mode of activation on a molecular structural level. The differential second derivative FTIR (SEDIR) and UV (SEDUV) protocols were developed and coupled with second derivatization to enhance the differences.[14] Namely, second derivatization of IR curves of a series of alkyl-susbstituted guanines leads to enhanced resolution over regular IR spectra. The basis of the differential spectral method is to subtract the spectrum of the mitosene moiety (the form of mitomycin in which the aziridine ring is cleaved) from that of a guanine/mitomycin monoadduct adduct, and to see which of the reference alkylguanine spectra resembles the difference spectrum most.

MC reacts with the dinucleoside phosphate d(GpC) or with DNA under mild acidic pH giving an extremely complex mixture of monosubstituted guanine adduct and N-formamidopyrimidines.[15] The structures were determined by collecting model compounds from the HPLC into -78□ flasks, quenching the interconversions by acetylation, and establishing their structures by SEDIR and SEDUV, etc.

A MC and DNA bifunctional adduct was believed to play a central role in its antitumor activity but had eluded isolation. An important finding was that sodium dithionite $Na_2S_2O_4$ yielded mainly the bisadduct. Elucidation of the various adduct structures led to rationalizations of their formation and the enhanced cytotoxicity of MC under hypoxic conditions.[13]

6. *Philanthotoxins (Figs. 6a, 6b).*

The venom of *Philanthus triangulum F.*, a digger wasp in the Sahara desert that preys on honeybees, blocks the

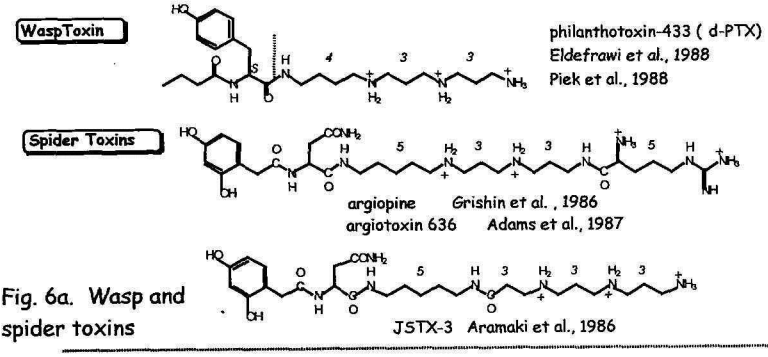
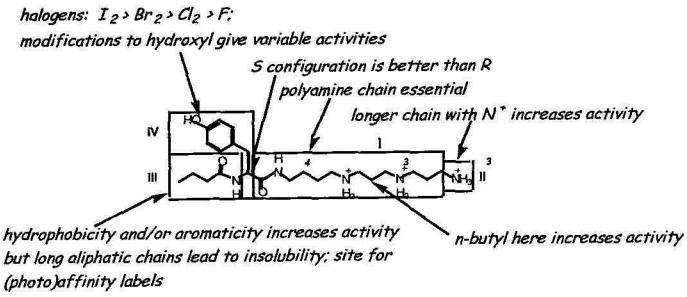


Fig. 6a. Wasp and spider toxins



These structure activity relationships reflect general trends in glutamate and nAChR.
Simultaneous modifications in: Regions II and IV are multiplicative or better, while Regions II and III are less than multiplicative.

Fig. 6b. SAR of philanthotoxins

When III = $n\text{-}C_9H_{19}CONH$-, further change reduces activity.

quisqualate-sensitive glutamate receptor (qGlu-R) and the nicotinic acetylcholine receptor (nACh-R). The most active component of the venom, philanthotoxin-433 (PhTX, numerals denote number of methylenes in the polyamine chain), was isolated from the female venom glands and its structure was determined as shown in Fig. 6a.[16,17] PhTX-433 is a non-competitive reversible antagonist of qGlu-R of invertebrate skeletal muscle and some vertebrate Glu-R. About 80 polyamine toxins have also been isolated from spider venoms and are potent antagonists of Glu-R and nACh-R.[18,19] Two of the spider toxins are shown in Fig. 6a.

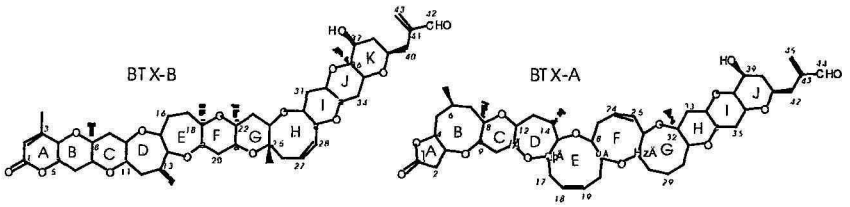


Fig. 7. Brevetoxins A and B.

Over 100 synthetic analogs resulted in SAR summarized in Fig. 6b.[20-22] Photoaffinity studies with nACh-R using radioactive bis-iodo(^{125}I) p-azidobenzamide analog led to a working stereo model showing the ligand / receptor interaction. Namely, the polyamine "tail" chain of PhTX can be lined against the hydrophilic rings in nAChR, while the Tyr and butyryl moieties of PhTX ("head" group) are in the cytoplasmic hydrophobic region. The distances between the PhTX amino groups are close to the 5.4 Å pitch of alpha helices of the receptor. A study with analogs containing large hydrophobic aromatic groups or porphyrins instead of the Tyr and butyryl groups suggested that the mode of binding is with the head groups in the hydrophobic cavity of the receptor. Recently the philanthotoxins haave been actively revisited.[23-25]

7. Brevetoxins (BTX) (Fig. 7).

A bloom of red tide toxin leads to massive fish deaths causing huge damage to mariculture. A culture (50 liter) of the dinoflagellate *Ptychodiscus brevis* Davis, the "red tide," yielded 90 mg of a crude mixture that yielded 0.8 mg BTX-A, 5 mg B and 0.4 mg C. The crystals of BTX-B that fortunately formed from MeCN in an NMR tube were submitted to X-ray studies (Jon Clardy) which disclosed the unprecedented ladder-like structure. The absolute configuration was determined from the CD of a glycol dibenzoate prepared from the 27-ene;[26] the absolute configuration was established by X-ray as well.[27] Studies with acetate doubly labeled with ^{13}C and the NMR INADEQUATE[28] and other new pulse sequences[29] as well as by Yuzuru Shimizu[30] have clarified its unique biosynthetic route. NMR and MS analyses and logical buildup led to a structure for BTX-A ($C_{49}H_{70}O_{13}$)[31] that differed from the X-ray structure[32] in the 6-Me configuration to which an alpha configuration was assigned. [31]

The toxicity of BTX-B is regarded to be due to its specific binding to voltage-sensitive sodium channels.[33] Sodium NMR[34] and CD[35] indicated that a further mechanism need be considered. Namely, BTX-B itself forms a cyclic channel superstructure in phosphatidylcholine lipid bilayers with ion selectivities of $Li^+ >> K^+ >> Cs^+$.[35]

8. Visual pigments (Fig. 8).

Our main protocol in studying mechanistic and structural aspects of rhodopsin (Rh), the visual pigment, is by incorporating synthetic retinal analogs[36] into the apoprotein.

8a. Rhodopsin (Rh)(Fig. 8a).

In Rh the 11-cis-retinal is bound to Lys-296 of opsin through a protonated Schiff base as in **1**. Upon irradiation the chromophore isomerizes to all-trans and after passing through several intermediates, it is expelled as all-trans retinal **2**. An external point charge model that placed a point negative charge arising from the opsin binding site near carbons 11 – 14 helped in understanding the participation of electrostatic interactions in regulating the absorption maxima of various pigments (1979).[37]

Pigments reconstituted from retinals with fixed double bonds, e.g., **3**, **4**, **5**: showed that isomerization is required for vision (1980),[38] bacteriorhodopsin proton pumping (1983)[39] and phototaxis (1991)[40] (with ret-5 **10**); provided the first chemical tool for mechanistic investigation of bleaching adaptation (1990, 1994) [41,42] (with ret-7 **4**); and led to the absolute sense of twist around the 6,7 single bond (2001)(with 6-s-locked **6**),[43] and biologically relevant conformation and the mode of entry of retinal (as shown by **8**) into Rh (2002)[44] (with cyclopropyl ret-7 **7**). Photolabeled retinals, e.g., diazoketone **9**, were used to locate the chromophore in Rh (1994),[45] and to trace the changes occurring in chromophore/binding site interactions throughout the visual transduction pathway, the first and so far only case for G protein coupled receptors (200O).[46]

8b. Ocular age pigment A2-E (Fig. 8b):

Age-related macular degeneration (AMD) is the leading cause of blindness in elderly people. The main fluorescent eye pigments that lead to AMD, A2E and iso-A2E were isolated from 250 over 40-year-old eyes and structure determined (1996, 1998).[47,48] The route and site of biosynthesis was also established (2000).[49] These fluorophores are a target for biomedical investigations of AMD. Blue light irradiation oxidizes A2E and introduces nine epoxide rings as shown, an unprecedented structure that is apoptotic (2001).[50]

9. Exciton chirality method for absolute configurational studies (Fig. 9)

The exciton chirality circular dichroic method (1969-)(ref. 5) is a nonempirical technique for determination of molecular chirality of practically any type of organic molecule, from small molecules to various biopolymers. An outline of the method is briefly described. The through space interaction of the electric transition moments of two chromophores gives rise to an exciton coupled "split" CD spectrum or "bisignate curve." In the case of the bis-p-substituted benzoate shown in Fig. 9, the absolute sense of twist of the two transition moments, which constitute a clock-wise chirality, give rise to the depicted bisignate CD which is defined as positive.

The method continues to develop into new areas with great versatility. A microscale method for oligosaccharide structure determination is under study by tagging the free hydroxyl groups and those originally involved in glycosidic linkages with different chromophores and comparing the characteristic CD with published reference curves. This CD methods requires no reference samples(1990).[51] The method is being further improved by combination with MS.
Porphyrins are promising chromophores for the CD exciton chirality method. Their Soret band absorptions around 400 nm is intense and removed from usual chromophores; they also have tendencies of stacking. These properties are utilized in

using them as reporter groups to determine absolute configurations of various substrates including carboxylic acids, alcohols and amines (1996-).[52,53]

Fluorescence detected CD (FDCD): Fluorophores used as chromophores in exciton coupled CD enhance the sensitivity 50 to 100-fold (1997).[54] The method is under further study.[55]

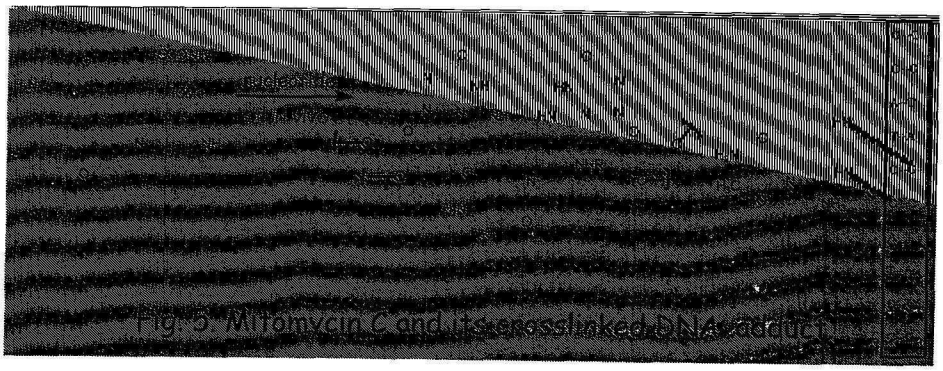


Fig. 5. Mitomycin C and its crosslinked DNA adduct

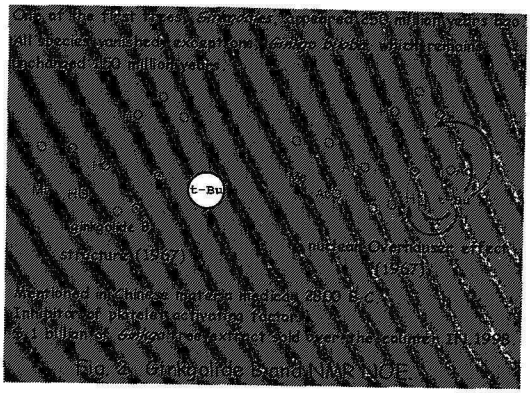


Fig. 6. Gymnodimine

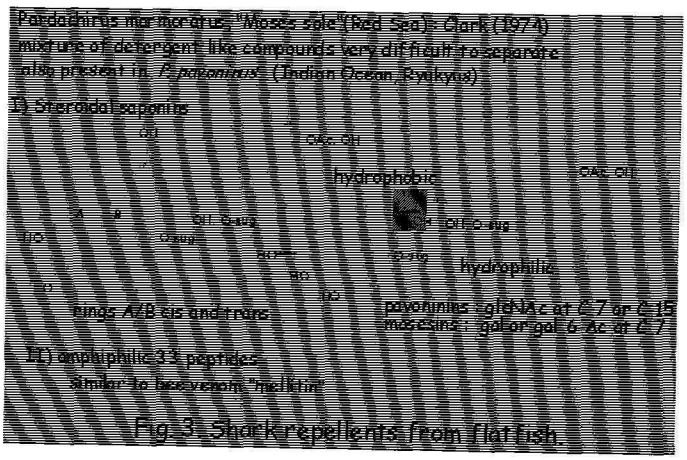
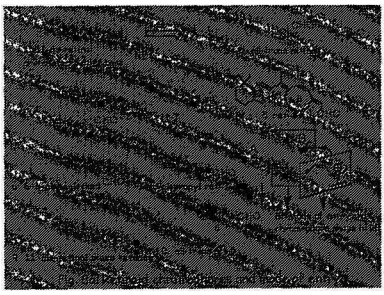


Fig. 3. Shark repellents from flatfish

Fig.4. Benzopyrene and its DNA adduct



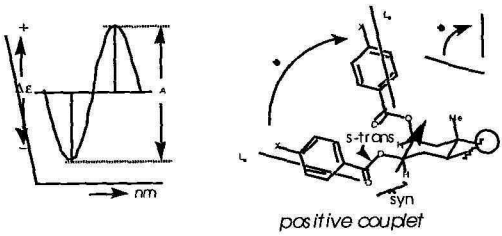


Fig. 9. Exciton chirality method.

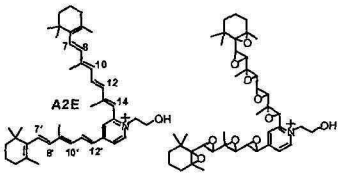


Fig. 8b. A2E and nonaoxirane

Selected References:

(1) Nakanishi, K.; Koreeda, M.; Sasaki, S.; Chang, M. L.; Hsu, H. Y. *Chem. Commun* **1966**, 915-917.

(2) Takemoto, T.; Ogawa, S.; Nishimoto, N. *Yakugaku Zasshi* **1967**, *87*, 325.

(3) Nakanishi, K. *Steroids* **1992**, *57*, 649-57.

(4) Naya, Y.; Kishida, K.; Sugiyama, M.; Murata, M.; Miki, W.; Ohnishi, M.; Nakanishi, K. *Experientia* **1988**, ***44***, 50-52.

(5) Naya, Y.; Miki, W.; Ohnishi, M.; Ikeda, M.; Nakanishi, K. *Pure & Appl. Chem.* **1989**, ***61***, 465-468.

(6) Nakanishi, K. *Pure Appl. Chem.* **1967**, *14*, 89-113.

(7) Stromgaard, K.; Saito, D. R.; Shindou, H.; Ishii, S.; Shimizu, T.; Nakanishi, K. *J. Med. Chem.* **2002**, 4038-4046.

(8) Tachibana, K.; Sakaitanai, M.; Nakanishi, K. *Science* **1984**, *226*, 703-5.

(9) Thompson, S. A.; Tachibana, K.; Nakanishi, K.; Kubota, I. *Science* **1986**, *233*, 341-3.

(10) Weinstein, I. B.; Jeffrey, A. M.; Jennette, K. W.; Blobstein, S. H.; Harvey, R. G.; Harris, C.; Autrup, H.; Kasai, H.; Nakanishi, K. *Science* **1976**, *193*, 592-5.

(11) Koreeda, M.; Moore, P. D.; Yagi, H.; Yeh, J.; H.J.C.; Jerina, D. M. *J. Am. Chem. Soc.* **1976**, *98*, 6720.

(12) Kasai, H.; Nakanishi, K.; Traiman, S. *J. Chem. Soc. Chem. Commun.* **1978**, 798-800.

(13) Tomasz, M.; Lipman, R.; Chowdary, D.; Pawlak, J.; Verdine, G. L.; Nakanishi, K. *Science (Washington, D. C.* **1987**, *235*, 1204-8.

(14) Verdine, G. L.; Nakanishi, K. *J. Am. Chem. Soc.* **1985**, *107*, 6118-20.

(15) Tomasz, M.; Lipman, R.; Lee, M. S.; Verdine, G. L.; Nakanishi, K. *Biochemistry* **1987**, *26*, 2010-27.

(16) Eldefrawi, A. T.; Eldefrawi, M. E.; Konno, K.; Mansour, N. A.; Nakanishi, K.; Oltz, E.; Usherwood, P. N. R. *Proc. Natl. Acad. Sci. U. S. A.* **1988**, *85*, 4910-13.

(17) Piek, T.; Fokkens, R. H.; Karst, H.; Kruk, A.; Lin, A.; van Marl, J.; Nakajima, T. N., N.M.M.; Shinozaki, H.; Spanjer, W.; Tong, Y. C. *Neurotox '88* **1988**, 61-76.

(18) Grishin, E. Y.; Volkova, T. M.; Arseniev, A. S.;

Reshetova, O. S.; Onoprienko, V. V.; Magazanik, L. G.; Antonov, S. M.; Federova, I. M. *Bioorg. Khim.* **1986**, *12*, 1121.

(19) Adams, M. E.; Carney, R. L.; Enderlin, F. E.; Fu, E. W.; Jarema, M. A.; Li, J. P.; Miller, C. A.; Schooley, D. A.; Shapiro, M. J.; Venema, V. J. *Biochem. Biophys. Res. Commun.* **1987**, *148*, 678-683.

(20) Nakanishi, K.; Choi, S. K.; Hwang, D.; Lerro, K.; Orlando, M.; Kalivretenos, A. G.; Eldefrawi, A.; Eldefrawi, M.; Usherwood, P. N. R. *Pure Appl. Chem.* **1994**, *66*, 671-8.

(21) Choi, S.-K.; Kalivretenos, A. G.; Usherwood, P. N. R.; Nakanishi, K. *Chem. Biol.* **1995**, *2*, 23-32.

(22) Huang, D.; Jiang, H.; Nakanishi, K.; Usherwood, P. N. R. *Tetrahedron* **1997**, *53*, 12391-12404.

(23) Stromgaard, K.; Brier, T. J.; Andersen, K.; Mellor, I. R.; Saghyan, A.; Tikhonov, D.; Usherwood, P. N. R.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *Journal of Medicinal Chemistry* **2000**, *43*, 4526-4533.

(24) Stromgaard, K.; Bjornsdottir, I.; Andersen, K.; Brierley, M. J.; Rizoli, S.; Eldursi, N.; Mellor, I. R.; Usherwood, P. N. R.; Hansen, S. H.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *Chirality* **2000**, *12*, 93-102.

(25) Stromgaard, K.; Mellor, I. R.; Andersen, K.; Neagoe, I.; Pluteanu, F.; Usherwood, P. N. R.; Krogsgaard-Larsen, P.; Jaroszewski, J. W. *Bioorganic & Medicinal Chemistry Letters* **2002**, *12*, 1159-1162.

(26) Lin, Y. Y.; Risk, M.; Ray, S. M.; Van, E. D.; Clardy, J.; Golik, J.; James, J. C.; Nakanishi, K. *J. Am. Chem. Soc.* **1981**, *103*, 6773-5.

(27) Shimizu, Y.; Bando, H.; Chou, H.-N.; van Dyne, G.; Clardy, J. *J. Am. Chem. Soc.* **1986**, 1656-1658.

(28) Lee, M. S.; Repeta, D. J.; Nakanishi, K.; Zagorski, M. G. *J. Am. Chem. Soc.* **1986**, *108*, 7855-6.

(29) Lee, M. S.; Qin, G.; Nakanishi, K.; Zagorski, M. G. *J. Am. Chem. Soc.* **1989**, *111*, 6234-41.

(30) Chou, H. N.; Shimizu, Y. *J. Am. Chem. Soc.* **1987**, *109*, 2184.

(31) Pawlak, J.; Tempesta, M. S.; Golik, J.; Zagorski, M. G.; Lee, M. S.; Nakanishi, K.; Iwashita, T.; Gross, M. L.; Tomer, K. B. *J. Am. Chem. Soc.* **1987**, *109*, 1144-50.

(32) Shimizu, T.; Chou, H.-N.; Bando, H.; van Dyne, G.; Clardy, J. *J. Am. Chem. Soc.* **1986**, *108*, 514.

(33) Gawley, R. E.; Rein, K. S.; Jeglitsch, G.; Adams, D. J.; Theodorakis, E. A.; Tiebes, J. H.; Nicolau, D. G.; Baden, D. G. *Chem. & Biol.* **1995**, *2*, 533.

(34) Matile, S.; Nakanishi, K. *Angew. Chem., Int. Ed. Engl.* **1996**, *35*, 757-9.

(35) Matile, S.; Berova, N.; Nakanishi, K. *Chem. & Biol.* **1996**, *3*, 379-392.

(36) Nakanishi, K.; Crouch, R. *Isr. J. Chem.* **1995**, *35*, 253-272.

(37) Honig, B.; Dinur, U.; Nakanishi, K.; Balogh, N. V.; Gawinowicz, M. A.; Arnaboldi, M.; Motto, M. G. *J. Am. Chem. Soc.* **1979**, *101*, 7084-6.

(38) Akita, H.; Tanis, S. P.; Adams, M.; Balogh, N. V.; Nakanishi, K. *J. Am. Chem. Soc.* **1980**, *102*, 6370-2.

(39) Fang, J. M.; Carriker, J. D.; Balogh, N. V.; Nakanishi, K. *J. Am. Chem. Soc.* **1983**, *105*, 5162-4.

(40) Derguini, F.; Mazur, P.; Nakanishi, K.; Starace, D. M.; Saranak, J.; Foster, K. W. *Photochem. Photobiol.* **1991**, *54*, 1017-21.

(41) Corson, D. W.; Cornwall, M. C.; MacNichol, E. F.; Jin, J.; Johnson, R.; Derguini, F.; Crouch, R. K.; Nakanishi, K. *Proc. Natl. Acad. Sci. U. S. A.* **1990**, *87*, 6823-7.

(42) Corson, D. W.; Cornwall, M. C.; MacNichol, E. F.; Tsang, S.; Derguini, F.; Crouch, R. K.; Nakanishi, K. *Proc. Natl. Acad. Sci. U. S. A.* **1994**, *91*, 6958-62.

(43) Fujimoto, Y.; Ishihara, J.; Maki, S.; Fujioka, Y.; Wang, T.; Furuta, T.; Fishkin, N.; Borhan, B.; Berova, N.; Nakanishi, K. *Chem. Eur. J.*, **2001**, *7*, 4198-4204.

(44) Fujimoto, Y.; Fishkin, N.; Pescitelli, G.; Decatur, J.; Berova, N.; Nakanishi, K. *J. Am. Chem. Soc.* **2002**, 7294-7302.

(45) Zhang, H.; Lerro, K. A.; Yamamoto, T.; Lien, T. H.; Sastry, L.; Gawinowicz, M. A.; Nakanishi, K. *J. Am. Chem. Soc.* **1994**, *116*, 10165-73.

(46) Borhan, B.; Souto, M. L.; Imai, H.; Shichida, Y.; Nakanishi, K. *Science* **2000**, 2209-2212.

(47) Sakai, N.; Decatur, J.; Nakanishi, K.; Eldred, G. E.

J. Am. Chem. Soc. **1996**, *118*, 1559-60.

(48) Parish, C.; Hashimoto, M.; Nakanishi, K.; Dillon, J.; Sparrow, J. *Proc. Natl. Acad. Sci., USA* **1998**, *95*, 14609-14613.

(49) Liu, J.; Itagaki, Y.; Ben-Shabat, S.; Nakanishi, K.; Sparrow, J. R. *J. Biol. Chem.* **2000**, 29354-29360.

(50) Ben-Shabat, S.; Itagaki, Y.; Jockusch, J. R.; Sparrow, J.; Turro, N.; Nakanishi, K. *Angew. Chem.* **2001**, *41*, 814-817.

(51) Harada, N.; Nakanishi, K. *Accounts Chem. Res.* **1972**, *5*, 257-63.

(52) Huang, S.; Nakanishi, K.; Berova, N. *Chirality* **2000**, ***12***, 237-255.

(53) Kurtan, T.; Nesnas, N.; Li, Y.-Q.; Huang, X.; Nakanishi, K.; Berova, N. *J. Am. Chem. Soc.* **2001**, *123*, 5962-5973.

(54) Dong, J.-G.; Wada, A.; Takakuwa, T.; Nakanishi, K.; Berova, N. *J. Am. Chem. Soc.* **1997**, *119*, 12024-12025.

(55) Nehira, T.; Parish, C.; Jockusch, S.; Turro, N.; Nakanishi, K.; Berova, N. *Journal of the American Chemical Society* **1999**, *121*, 8681-8691.

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