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SUMMARY STATEMENT
(Privileged Communication)

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Application Number: 1 R01 CA0000717-01

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Review Group: ZCA1 RLB-7 (04)
NCI SPECIAL EMPHASIS PANEL

Meeting Date: JUNE 1997
Council: SEPT/OCT 1997
Requested Start: 09/01/1997
PCC: 00XX

Project Title: CYCLOOXYGENASE-2 INHIBITORS & COLON CANCER

SRG Action: Priority Score: 250
Human Subjects: 44-Human subjects involved - SRG concerns
Animal Subjects: 10-No live vertebrate animals involved for competing appl.
Gender: 1A-Both genders, scientifically acceptable
Minority: 1A-Minorities and non-minorities, scientifically acceptable
Children: 3U-No children included, scientifically unacceptable
Clinical Research - not NIH-defined Phase III Trial

Project Year	Direct Costs Requested	Estimated Total Cost
1	879,994	3,691,222
2	909,293	3,700,239
3	630,174	2,998,335
4	886,182	4,160,815
5	871,616	4,268,360
TOTAL	4,177,259	18,818,971

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by Institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

1 R01 CA 000717-01 MULDOON, T**INCLUSION OF CHILDREN PLAN UNACCEPTABLE****PROTECTION OF HUMAN SUBJECTS UNACCEPTABLE**

RESUME AND SUMMARY OF DISCUSSION: This is a multi center trial consisting of 14 institutions and 990 subjects. The primary goal is to test a cyclooxygenase-2 (COX-2) inhibitor (RK42B) for its effectiveness in reducing colonic polyp recurrence. Other goals are to investigate whether celecoxib reduces prostaglandin E2 content and alters cell proliferation and apoptosis in "normal" colonic mucosa; to determine whether the pharmacokinetics of - RK42B can predict the reduction of colonic PGE2; and to define potential mechanisms of polyp recurrence after prolonged Cox-2 inhibition. This is an excellent group of investigators who are tackling an important problem: the chemoprevention of colon carcinogenesis. They are well positioned to conduct a multi center trial of this magnitude. However, enthusiasm for this trial is diminished by several factors. Further animal experiments comparing RK42B to other NSAIDS with respect to tumor development are needed. Although the investigators have the ability to conduct such a study, the study as presented is considered premature. They have eliminated several of the intermediate studies that need to be conducted. Scoring of this good to very good application is recommended.

DESCRIPTION (provided by applicant): A pivotal, multicenter trial is proposed to test the hypothesis that RK42B a selective cyclooxygenase-2 inhibitor, will reduce colonic polyp recurrence and will ultimately delay or prevent colonic epithelial transformation. The Specific Aims of this pivotal trial are 1) To determine whether RK42B reduces the recurrence of adenomatous colonic polyps in humans; 2) To investigate whether RK42B reduces prostaglandin E2 (PGE2) content and alters cellular proliferation and apoptosis in morphologically normal colonic mucosa; 3) To determine whether pharmacokinetics of Rk42B can predict reduction of colonic PGE2 4) To define potential mechanisms of polyp recurrence after prolonged Cox-2 inhibition.

Healthy human subjects who have undergone a clearing colonoscopy with the removal of all polyps and at least a single polyp of = or 1cm in size or two or more polyps = or > 0.6 cm in size will be eligible for this trial. After a 1 month run-in period, subjects will be randomized to receive RK42B 200 mg twice daily, RK42BL 400 mg twice daily or placebo for 3 years. During the run-in period, a subset of subjects will undergo flexible sigmoidoscopy with 8 biopsies of morphologically normal colon mucosa. After 6 months of RK42B treatment, the subject subset will undergo a repeat flexible sigmoidoscopy with 8 normal tissue biopsies. At the end of 3 years treatment, all subjects will undergo diagnostic colonoscopy with detection and removal of any recurrent polyps and biopsy of morphologically normal colonic epithelial. Drug effect upon cyclooxygenases will be assessed from frozen polyps and normal biopsies. Proliferative activity in polyps and normal mucosa will be quantified by computed algorithmic quantitation of epithelial amaranthin labeling of colorectal mucin and Ki67. Apoptotic activity in polyps will be assessed using an apoptotic index as measured by the Apotag assay. p53 and kRas from polyps will be assessed by computed algorithmic immunohistochemistry and by mutational analysis. We base our sample size upon a conservative assumption that 25% of control subjects will have an adenomatous polyp upon reexamination. To detect a 50% reduction in polyp incidence after 3 years of RK42B treatment at a significance level of 0.05 and a power of 0.90, 990 subjects will be randomized.

COLLABORATING INSTITUTIONS: 12 sites in USA and 2 in Europe

(This critique includes the minimally edited review comments of individual reviewers, some of which may not be completely consistent with the overall merit rating. The numerical score should be considered the most accurate representation of the review outcome:)

CRITIQUE 1:

SIGNIFICANCE: Before a trial of this magnitude is undertaken we need information in four major areas: (1) that there is a strong scientific rationale for the intervention; (2) that the selection of intermediate markers is appropriate and justified; (3) that the experimental design is appropriate and in place; (4) that the investigators are qualified to implement the experimental design. This review will focus on these four areas.

The rationale behind the proposed studies is that colon cancer is a major public health issue in the United States today, and that chemoprevention may have a major impact on reducing the incidence of this disease. Nonsteroidal anti-inflammatory agents (NSAIDS) are protective against colon cancer, purportedly by inhibiting cyclooxygenase activity which in turn decreases the production of prostaglandins. There are two cyclooxygenase enzymes, COX-1 is constitutively expressed whereas COX-2 is inducible and thought to be induced as part of the tumorigenic process. Thus, the hypothesis is that a specific inhibitor of COX-2 rather than COX-1 should be more efficacious in reducing colon cancer risk. The drug to be tested, RK42B, is a specific inhibitor of COX-2. This rationale (stated above) forms the basis of the choice of intervention agent, the study population, the major endpoints, and the biological markers to be measured, thus it will be critiqued in some detail.

There is ample evidence that colon cancer is a major public health issue in the United States today, and there is good justification to believe that chemoprevention may have a major impact on the incidence of this disease. Recent data accumulated from both epidemiological studies and animal trials strongly suggests that NSAIDS reduced the incidence of colon cancer. In fact, most studies, including one that analyzed data from 662,424 North American adults over a seven year period, showed a significant reduction in colorectal cancer risk. However, it should be noted (and should have been included in this application) that in the one available randomized trial, in which 22,071 male physicians were enrolled in 1982, no effect of assignment to take 325 mg of aspirin every other day against colorectal cancer or polyps was found (Gann et al., 1993 and a follow up reported by Strumer et al., 1996). Although the mechanism behind the purported protective effect is not entirely clear, a reasonable hypothesis is that at least one factor may involve reduction of prostaglandin production. Prostaglandin E2 has been shown to be higher in certain tumor tissue as compared to that from healthy controls. Further, there are some data (although not all are in agreement) that PGE2 increases colonic cell proliferation, and generally agents that increase cell proliferation are promoters of tumorigenesis. Recent data also suggests that COX-2, unlike COX-1 is induced and that this induction coincides with initiation or progression of the transformed phenotype. Thus, in theory, the rationale behind using a specific COX-2 inhibitor is appropriate and testable.

It is still an open question as to whether or not the protective effect of NSAIDS against colon tumorigenesis is mediated through a decrease in prostaglandin production. For example, in one experimental colon cancer study, Craven et al. found that 1,2-DMH carcinogenesis was not closely related to inhibition of PGF2 production, but rather to suppression of COX mediated metabolic activation of 1,2-DMH, suggesting that NSAIDS work at the level of initiation, rather than promotion. Concentrations of NSAIDS required to inhibit cell growth appear to be much greater than those required to inhibit cyclooxygenase, and sulindac sulfone that does not inhibit PG synthesis is equally effective in inhibiting chemical carcinogenesis and growth of tumor cell lines. These results suggest that the antitumorigenic effects of NSAIDS may be mediated through both cyclooxygenase dependent and independent pathways. There are two other aspects of the investigators' hypothesis that are not clearly justified by the literature. One is that specific inhibition of COX-2 should be more protective against colon carcinogenesis than global inhibition of both COX enzymes. Although the P1 presents experimental data from the literature in support of the hypothesis that induction of COX-2 is more closely related to future tumor development than is overexpression of COX-1, the studies that show a protective effect of NSAIDS, particularly aspirin, are on inhibitors of both enzymes. It appears that all NSAIDS are protective against colon tumorigenesis, even though they may work by different mechanisms. Why there should be a special emphasis on this drug, when aspirin confers a 50% reduction in risk of colorectal cancer occurrence is not adequately justified. Other agents have also

been shown to be protective include sulindac, ibuprofen, indomethacin, piroxicam and ketoprofen. There are no clinical or experimental data showing a greater protective effect of specific COX-2 inhibitors over inhibitors of both enzymes. Thus there is no apparent justification for testing a specific COX-2 inhibitor, and no clear rationale as to why RK42B should be better than other NSAIDS.

To the best of this reviewer's knowledge (and, as indicated in the proposal) there are very few studies on RK42B. All of the human data are described in an investigational brochure from the company, RK42B and were not available in the Appendix or otherwise available for review. In the Appendix it is reported that there is an ongoing long term safety trial with a total of 2,319 subjects enrolled and treated at 400 mg of RK42B1 twice daily for treatment of arthritis. Results of this trial are not yet available. There are two reports on RK42B~ in the literature. In one (Seibert et al., 1995) RK42B was compared to indomethacin as an inhibitor of Cox-1 and Cox—2 in SF9 cells expressing human Cox-1 and Cox-2. Whereas indomethacin's IC50 was similar for both enzymes, RK42B had an IC50 of 0.04 microM for Cox-2 and 15 microM for Cox-1, showing that it is a much greater inhibitor of Cox-2 than Cox-1. The other published study was in male F344 rats on aberrant crypt formation (Reddy et al., 1996), 'in which aberrant crypt formation was reduced at administration of RK42B 1,500 ppm, but not at 150 ppm, as compared to the placebo control. The 1,500 ppm RK42B was similar to results from 320 ppm sulindac. In an addendum to this application the P1 reports that the rat study has now evaluated adenomas and carcinomas as end points, and that RK42B reduced the number of adenomas and carcinomas from 91% in placebo controls to 25% in treated animals. However, these data have not yet been published and there are no published human studies to evaluate. No data are available on the dose of RK42B to decrease prostaglandin production, or whether or not it inhibits COX-2 activity in vivo.

APPROACH: The markers chosen for this intervention are colonic polyp recurrence; prostaglandin E2 content in colonocytes; measurements of cell proliferation and apoptosis in colonocytes; pharmacokinetics of RK42B and pretreatment aneuploidy, expression of kRas; and expression of mutated p53. The aneuploidy, kRas and p53 measurements are to explain possible reasons behind why a subject was unresponsive to RK42B treatment.

The use of polyp recurrence as an intermediate marker is well justified by the authors and by the literature. This is an appropriate endpoint, and three years post intervention is an appropriate time to measure this endpoint. The measurement of PGE2 is an important and necessary one, since the hypothesis is that RK42B works, in part, by inhibiting PGE2 production. Whether or not changes in cell proliferation and/or apoptosis will correlate with RK42B treatment, polyp -recurrence and prostaglandin inhibition is an unknown, and not nearly as predictable as the literature used to suggest, but nevertheless these measurements should be taken, if only to exclude their merit as predictive markers. The measurement of pretreatment aneuploidy, kRas and p53 is of interest as baseline data to use in evaluating "nonresponders".

As noted above, a major hypothesis by the investigators is that RK42B should be protective due to inhibiting COX-2, decreasing PGE2 production which in turn should decrease cell proliferation. However, the investigators' own data do not support an inhibition of cell proliferation. In fact, in their aspirin trial (described in the preliminary data section) aspirin administration, while decreasing PGE2 production, did not decrease cell proliferation. In a study by Craven in rats treated with aspirin, PGE2 levels in colonic mucosa were decreased, but cell proliferation was actually enhanced. Further, in the phase I trial of sulindac sulfone also described in the preliminary data section, the intervention actually increased cell proliferation in polyps taken from patients treated with the drug. Thus, it is unlikely that a decrease in cell proliferation will prove to be an acceptable marker or substitute for decreased polyp recurrence in this intervention. Nevertheless it is important to measure these changes as they provide information necessary to the overall mechanistic hypothesis. In summary, the markers are of interest but not for the reasons outlined in the RFA. None of these markers is likely to be of use as an endpoint in itself as a measurement of a protective effect of an intervention that predicts for the later lowered incidence of colon cancer.

The investigators have chosen an appropriate target population, those at high risk for colon cancer (presenting with a single polyp of > 1 cm in size or two or more polyps > 0.6 cm in size. The intervention period (3 years) is appropriate to assess recurrence of polyps. Investigators are experienced in clinical trials, the assays are generally in house and the procedures for accruing participants, protecting privacy, analyzing data and dispensing with samples are in place. The investigators are to be commended on their attention to detail in terms of diet analysis, exclusion criteria, data handling and analysis. They have clearly thought through the logistics of such a multi center trial. The institutional environment in which the research is conducted, including the availability of space, equipment and patients as well as the physical proximity of participants is appropriate. The power calculations are appropriate and

The statistical design and mechanism for the management and verification of research data is appropriate. Methods for data and tissue collection and analysis are in place. The bioanalytical procedures are well documented, and the investigators have prior experience in the endpoints to be evaluated. Adequate attention is paid to involvement of NCI Program staff with the proposed research.

INNOVATION: There are innovative uses of technology that the investigators have clearly learned from experience. For example, adherence monitoring is well worked out by the investigators with the electronic monitoring of the blister pack. The use of a different model for cell proliferation and apoptosis is of interest and is well documented.

INVESTIGATORS: The Principal Investigator is an established scientist with a substantial record of independent research. The PI has selected an excellent group of investigators to conduct this trial. The PI and staff at the University of Punxatawney Cancer Prevention Program are experienced in multicenter clinical trials. Dr. Muldoon is PI of a contract funded chemoprevention trial for bladder cancer with fenretinide. The University of Punxatawney Prevention Program serves as the statistical and data management office' for a multicenter chemoprevention trial of DFMO and Dr. Muldoon is PI of that trial. The time commitment of 20% by Dr. Muldoon for a trial of this complexity is on the low side, but somewhat ameliorated by the excellent staff he has assembled and the co-investigators on the project.

ENVIRONMENT: This is an international, multicenter trial of 12 clinical institutions, an administrative center, a Statistical and Data Management Office and a Central Laboratory.

OVERALL EVALUATION: In this reviewer's opinion, the data on RK42B that are currently available are insufficient to warrant a multi center clinical trial of 3 year intervention, and 5 year duration at this time. Several studies are required before such a trial would be warranted including additional data from animal studies showing equal or better ability to lower experimentally induced colon tumorigenesis as compared to other NSAIDS. Also needed is a short term human trial showing that PGE2 production is decreased at the dose suggested for the long term trial. Since the long term toxicity study is ongoing, results from this study would also be helpful.

PROTECTION OF HUMAN SUBJECTS FROM RESEARCH RISKS: Acceptable risk and adequate protection. The logistics of actually conducting the trial, and relating the subset trials to the overall trial need to be put in one section of the application. As it now stands one has to consult the application, appendices, individual letters from co-investigators noting their roles in the study, and the addendum to the protocol. It is unclear from the application itself as to how many subjects are anticipated from each site and what the likelihood is of that particular site recruiting and retaining these subjects. Information on involvement with NCI Program staff in the proposed research, although adequate, is not extensively described, nor are specifics provided for the data monitoring committee and how it will function.

GENDER, MINORITY AND CHILDREN SUBJECTS: G1A, M1A, C3U – Figures are not offered on previous accrual and retention rates for clinical trials conducted by the PI. Such data, in tabular form, would be helpful. There is no justification for the exclusion of children provided.

ANIMAL WELFARE: No animal studies are proposed.

BUDGET: Appropriate.

CRITIQUE 2:

SIGNIFICANCE: This is a multicenter trial including sites, requiring the shipment of large numbers of fresh frozen tissue samples and plasma on dry ice to centers in Mid State (central laboratory) and ASMU (micronutrient laboratory). No data are presented to support feasibility or quality control of tissue sample acquisition either within or outside the US. Furthermore, there is no information in this application to indicate the feasibility of the specimen tracking system or database, which is a well documented problem in several ongoing NCI multi-center US translational chemoprevention trials. These aspects will be critical to the monitoring of this logistically complex proposal involving tissue and plasma acquisition, shipping, analysis and storage from 12 US centers and 2 international centers. This consortium of 14 centers does not have a proven record of working together on any studies. It is extremely ambitious to attempt a clinical trial of this magnitude and complexity including potentially problematic issues such as shipping specimens internationally on dry ice.

No data is presented to support estimated accrual at the individual centers. Curiously, nine centers show an estimated enrollment of 48 patients per center. How were these precise figures derived? For example, there should be a breakdown of numbers of patients potentially eligible (which should be based on actual data available from each institution). In turn, this number could be used to estimate the number of patients enrolled, which is classically 10-20% for a placebo controlled study such as that proposed.

APPROACH: There are very few details provided on analysis of biomarkers (three general sentences). The authors need to give more data on expected baseline expression and expected effects of intervention as well as more detailed plans for analyzing each biomarker. It is stated that the baseline "markers" will be analyzed by linear or non-linear models and changes by parametric or nonparametric models. This description is too general and not helpful for this proposal which plans to use several different marker assays (e.g., EIA, Western blot, immunohistochemistry and mutational analyses using PCR/SSCP) and quantitation methods to analyze at least seven biomarkers (COX-2 protein levels, Ki-67, K-Ras, cyclooxygenase, apoptosis, p53, PGE-2) which have very different expression patterns. Furthermore, the application indicates that some of these markers will only be performed on a subset of subjects and in some cases will involve marker studies in normal as well as polyp tissue. The above issues present complex statistical analytical problems which need to be addressed in greater detail. There is extensive published work regarding proliferation markers in colon cancer chemoprevention and, therefore, it would be important to prospectively indicate the proposed analysis of the proliferation marker Ki-67. It would also be important to indicate the planned analyses of other markers based on supporting data from the literature and preliminary data (which presumably lead to selection of these markers) including which markers will be analyzed as discrete vs. continuous variables.

INNOVATION: The proposed studies focus on a novel specific inhibitor of COX-2, RK42B.

INVESTIGATORS: The applicants are highly qualified.

ENVIRONMENT: Adequate

OVERALL EVALUATION: This application is rated very good to good.

PROTECTION OF HUMAN SUBJECTS FROM RESEARCH RISKS: There is almost no risk to subjects from the research proposed in this application. This issue is very well described in the application. Although there is a Data Safety and Monitoring Committee identified, the structure of the

statistical considerations does not tell us just when they will be approached, when they will be invoked and what they are empowered to do. More detail is needed.

GENDER, MINORITY AND CHILDREN SUBJECTS: G1A, M1A, C3U- There are no exclusions of the basis of sex or race, and the PI predicts a slight predominance of males over females because of a somewhat higher incidence of colorectal carcinoma in males and because VA Medical Centers will be used to solicit subjects. They provided ethnic background data for the University of Punxatawney. They will specifically include the Gopher Hospital in Sunnyside in order to enhance minority accrual to trials. The other issue regards the participation of children. We know that children, adolescents who would fit the NIH defined criteria of being under age 21, do have a risk for colon cancer and this does exclude the known familial types of colon cancer; this is not adequately addressed. In fact, it needs to be indicated clearly that subjects in the pediatric age group would be eligible for this study, or adequate justification why they should be excluded.

BUDGET: The budget is very complicated and although very large appears to be well justified as requested. However, it is beyond the capabilities of this committee to critically evaluate and should this grant be funded, the appropriate NCI staff are to verify that this budget is appropriate. The five year budget is approved.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW ADMINISTRATOR TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS (Resume): UNACCEPTABLE

The committee expressed concern that although a Data Safety and Monitoring Committee was described in the application, the description of when they would be approached, when they would be invoked and what they would be empowered to do was inadequate and that more detail should be provided.

INCLUSION OF WOMEN PLAN (Resume): ACCEPTABLE

G1A – The committee felt that an adequate number of women would be included in the proposed studies.

INCLUSION OF MINORITIES PLAN (Resume): ACCEPTABLE

M1A – The committee felt that an adequate number of minorities would be included in the proposed studies.

INCLUSION OF CHILDREN PLAN (Resume): UNACCEPTABLE

C3U – The committee felt that the exclusion of children from the proposed studies was unacceptable as no justification for their exclusion was provided.

VERTEBRATE ANIMAL (Resume): NOT APPLICABLE

COMMITTEE BUDGET RECOMMENDATIONS: The budget was recommended as requested.
(Roster not included for this mock study section)

NOTICE: The NIH has modified its policy regarding the receipt of amended applications. Detailed information can be found by accessing the following URL address:
<http://grants.nih.gov/grants/policy/amendedapps.htm>