Final Progress Report for Research Projects Funded by Health Research Grants

Instructions: Please complete all of the items as instructed. Do not delete instructions. Do not leave any items blank; responses must be provided for all items. If your response to an item is "None", please specify "None" as your response. "Not applicable" is not an acceptable response for any of the items. There is no limit to the length of your response to any question. Responses should be single-spaced, no smaller than 12-point type. The report **must be completed using MS Word**. Submitted reports must be Word documents; they should not be converted to pdf format. Questions? Contact Health Research Program staff at 717-783-2548.

1. **Grantee Institution:** The Pennsylvania State University

2. **Reporting Period (start and end date of grant award period):** 1/1/2009 – 12/31/2012

3. **Grant Contact Person (First Name, M.I., Last Name, Degrees):** John Anthony, MPA

4. **Grant Contact Person's Telephone Number:** 814-935-1081

5. **Grant SAP Number:** # 4100047645

6. **Project Number and Title of Research Project:** 32 - Efficacy of Gemcitabine for Pancreatic Cancer: Role of DNA Polymerases

7. **Start and End Date of Research Project:** 07/08/2009 – 12/31/2012

8. **Name of Principal Investigator for the Research Project:** Thomas E. Spratt, PhD

9. **Research Project Expenses**.

   9(A) Please provide the total amount of health research grant funds spent on this project for the entire duration of the grant, including indirect costs and any interest earned that was spent:

   $138,642

   9(B) Provide the last names (include first initial if multiple individuals with the same last name are listed) of **all** persons who worked on this research project and were supported with health research funds. Include position titles (Principal Investigator, Graduate Assistant, Post-doctoral Fellow, etc.), percent of effort on project and total health research funds expended for the position. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project	Cost
Gowda	Post-doc	50% YR1	$20,234.76
Lin	Asst Prof	13.7% YR1;	$13,332.93

9(C) Provide the names of **all** persons who worked on this research project, but who *were not* supported with health research funds. Include position titles (Research Assistant, Administrative Assistant, etc.) and percent of effort on project. For multiple year projects, if percent of effort varied from year to year, report in the % of Effort column the effort by year 1, 2, 3, etc. of the project (x% Yr 1; z% Yr 2-3).

Last Name	Position Title	% of Effort on Project
Spratt	PI	5
Jiang	Co-PI	2
Ahn	biostatistician	2
Ryder	Clinical coordinator	5
Escobar	Clinical coordinator	2

9(D) Provide a list of **all** scientific equipment purchased as part of this research grant, a short description of the value (benefit) derived by the institution from this equipment, and the cost of the equipment.

Type of Scientific Equipment	Value Derived	Cost
Qtrap 4000 MS/MS (Maintenance)	Provided optimized instrument for dozens of assays for investigators in 10 Basic Science and Clinical departments	$55,923

10. Co-funding of Research Project during Health Research Grant Award Period. Did this research project receive funding from any other source during the project period when it was supported by the health research grant?

Yes_____ No__X_____

If yes, please indicate the source and amount of other funds:

11. Leveraging of Additional Funds

11(A) As a result of the health research funds provided for this research project, were you able to apply for and/or obtain funding from other sources to continue or expand the research?

Yes_____ No__X_____

If yes, please list the applications submitted (column A), the funding agency (National Institutes of Health—NIH, or other source in column B), the month and year when the

application was submitted (column C), and the amount of funds requested (column D). If you have received a notice that the grant will be funded, please indicate the amount of funds to be awarded (column E). If the grant was not funded, insert "not funded" in column E.

Do not include funding from your own institution or from CURE (tobacco settlement funds). Do not include grants submitted prior to the start date of the grant as shown in Question 2. If you list grants submitted within 1-6 months of the start date of this grant, add a statement below the table indicating how the data/results from this project were used to secure that grant.

A. Title of research project on grant application	B. Funding agency (check those that apply)	C. Month and Year Submitted	D. Amount of funds requested:	E. Amount of funds to be awarded:
None	☐NIH ☐ Other federal (specify:_____) ☐ Nonfederal source (specify:_)		$	$

11(B) Are you planning to apply for additional funding in the future to continue or expand the research?

Yes_____ No____X_____

If yes, please describe your plans:

12. Future of Research Project. What are the future plans for this research project?

The results of the study were not promising enough to continue this research.

13. New Investigator Training and Development. Did students participate in project supported internships or graduate or post-graduate training for at least one semester or one summer?

Yes___X_____ No_____

If yes, how many students? Please specify in the tables below:

	Undergraduate	Masters	Pre-doc	Post-doc
Male				1
Female				
Unknown				
Total				**1**

	Undergraduate	Masters	Pre-doc	Post-doc
Hispanic				
Non-Hispanic				1
Unknown				
Total				**1**

	Undergraduate	Masters	Pre-doc	Post-doc
White				
Black				
Asian				1
Other				
Unknown				
Total				**1**

14. Recruitment of Out-of–State Researchers. Did you bring researchers into Pennsylvania to carry out this research project?

Yes_____ No____X_____

If yes, please list the name and degree of each researcher and his/her previous affiliation:

15. Impact on Research Capacity and Quality. Did the health research project enhance the quality and/or capacity of research at your institution?

Yes___X_____ No_____

If yes, describe how improvements in infrastructure, the addition of new investigators, and other resources have led to more and better research.

The serious recruitment problem experienced in this project contributed to the recognition that the institution needed to review and improve mechanisms available to investigators for estimating the numbers of patients who meet the eligibility criteria for clinical studies. That review was recently completed and the recommended changes are in the process of being implemented to improve the effectiveness and efficiency of various clinical trials operations through the development of more centralized administrative controls, including a major new data warehouse initiative to make reliable data on patient eligibility much more readily available to investigators who are planning new clinical trials.

16. Collaboration, business and community involvement.

16(A) Did the health research funds lead to collaboration with research partners outside of your institution (e.g., entire university, entire hospital system)?

Yes_____ No___X_____

If yes, please describe the collaborations:

16(B) Did the research project result in commercial development of any research products?

Yes_____ No___X_____

If yes, please describe commercial development activities that resulted from the research project:

16(C) Did the research lead to new involvement with the community?

Yes_____ No_____X_____

If yes, please describe involvement with community groups that resulted from the research project:

17. Progress in Achieving Research Goals, Objectives and Aims.

List the project goals, objectives and specific aims (as contained in the grant agreement). Summarize the progress made in achieving these goals, objectives and aims for the period that the project was funded (i.e., from project start date through end date). Indicate whether or not each goal/objective/aim was achieved; if something was not achieved, note the reasons why. Describe the methods used. If changes were made to the research goals/objectives/aims, methods, design or timeline since the original grant application was submitted, please describe the changes. Provide detailed results of the project. Include evidence of the data that was generated and analyzed, and provide tables, graphs, and figures of the data. List published abstracts, poster presentations and scientific meeting presentations at the end of the summary of progress; peer-reviewed publications should be listed under item 20.

This response should be a DETAILED report of the methods and findings. It is not sufficient to state that the work was completed. Insufficient information may result in an unfavorable performance review, which may jeopardize future funding. If research findings are pending publication you must still include enough detail for the expert peer reviewers to evaluate the progress during the course of the project.

Health research grants funded under the Tobacco Settlement Act will be evaluated via a performance review by an expert panel of researchers and clinicians who will assess project work using this Final Progress Report, all project Annual Reports and the project's strategic plan. After the final performance review of each project is complete, approximately 12-16 months after the end of the grant, this Final Progress Report, as well as the Final Performance Review Report containing the comments of the expert review panel, and the grantee's written response to the Final Performance Review Report, will be posted on the CURE Web site.

There is no limit to the length of your response. Responses must be single-spaced below, no smaller than 12-point type. If you cut and paste text from a publication, be sure symbols print properly, e.g., the Greek symbol for alpha (α) and beta (ß) should not print as boxes (□) and include the appropriate citation(s). DO NOT DELETE THESE INSTRUCTIONS.

Efficacy of Gemcitabine for Pancreatic Cancer: Role of DNA Polymerases – Pancreatic ductal adenocarcinoma is a very aggressive malignancy that is treated by gemcitabine. Its efficacy is thought to be due to inhibition of DNA synthesis. It is our hypothesis that newly discovered DNA polymerases modulate gemcitabine toxicity and thereby decrease its efficacy. The purpose of the present experiments is to test the hypothesis that inter-individual differences in the levels of these polymerases affect incorporation of gemcitabine into the DNA and its efficacy in pancreatic cancer patients receiving gemcitabine.

The goal of the project was to obtain blood cells from individuals with pancreatic cancer who have also received gemcitabine. We would collect blood 6 and 24 h after gemcitabine administration. The white blood cells would be isolated and (1) the gemcitabine content in the DNA would be measured by a HPLC-MS method, (2) the levels of DNA polymerases would be measured by Western analysis, and (3) the prognosis of the patient would be accessed.

This goal was not accomplished because of a lack of patient accrual into the study. Dr Jiang, identified approximately 20 patients as potential participants, of which only 2 gave blood.

Several changes in the protocol were made in order to accrue more patients.

1. The Study was opened to more physicians including Drs Belani, Cream, Harvey, Yee, and Almokadem. Unfortunately, these physicians did not identify any patients who would be potential participants. During this time period of approximated 6 months, approximated 10 patients were identified by Dr Jiang, none of whom decided to participate in the study.

2. The study was opened to other cancers that are treated with gemcitabine including lung, breast and colon. During this time period of about 6 months, Dr Jiang identified approximately five potential participants, none of whom participated in our study.

3. We determined that the time line of blood donation was too onerous for the patients. We first modified the protocol for a blood donation time of immediately and six hours after gemcitabine administration. Subsequently, we changed the protocol for right before and two hours after administration. Dr Jiang identified about five potential participants, none of whom gave blood.

None of these changes increased patient accrual. Thus, we did not accomplish this part of the study.

Two biochemical studies were performed in parallel to these clinical studies to evaluate our hypothesis.

1. We tested the hypothesis that base excision repair can bypass the inhibition of gemcitabine has on DNA replication. Specifically, we examined (1) the DNA polymerase beta catalyzed incorporation of gemcitabine triphosphate into DNA and (2) the subsequent ligation catalyzed by DNA ligase III / XRCC1. The rate of reaction would give an indication of whether gemcitabine can be incorporated into the DNA by this mechanism.

2. We tested the hypothesis that examined the levels of DNA polymerases in culture cells affect the extent by which gemcitabine is incorporated into the DNA of those cells. Specifically, we measured (1) the amounts of gemcitabine incorporated into the DNA, and (2) the levels of specialized DNA polymerases in several cell lines. A high correlation between incorporation and DNA polymerase levels would support the hypothesis.

1. Incorporation of dFdC into DNA during Base Excision Repair.

Reactivity of DNA polymerase beta with dFdC.

The rationale for these experiments is illustrated in Figure 1. We anticipated that dFdC might be incorporated during base excision repair because this synthesis would not be affected by the inhibition of DNA synthesis typically associated with dFdC. As shown below (left), when dFdC is incorporated, the next dNTP is incorporated readily, but the subsequent incorporation is inhibited. During BER, (right), this phosphodiester bond exists and does not have to be synthesized.

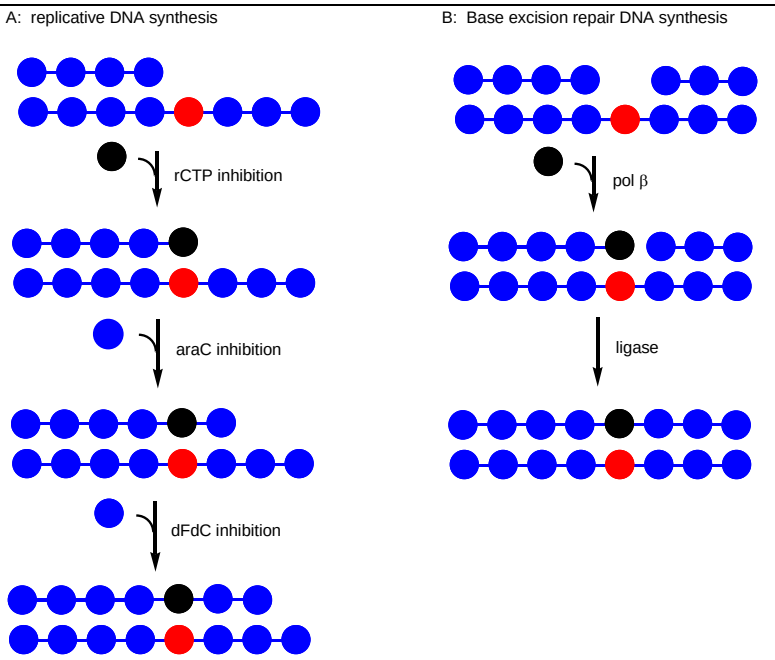

Figure 1. Blue circles represents any nucleotide, black represents a dG, and red dFdC. A. Incorporation of dFdC by replicative polymerases. B. Incorporation of dFdC during BER.

The steady-state and pre-steady-state kinetic parameters were obtained for the DNA polymerase beta incorporation of the triphosphates of dC, rC, FdC, araC, and dFdC (structures shown in Figure 2) opposite template dG. Two different gapped duplex were used as the substrate, as shown in Figure 3.

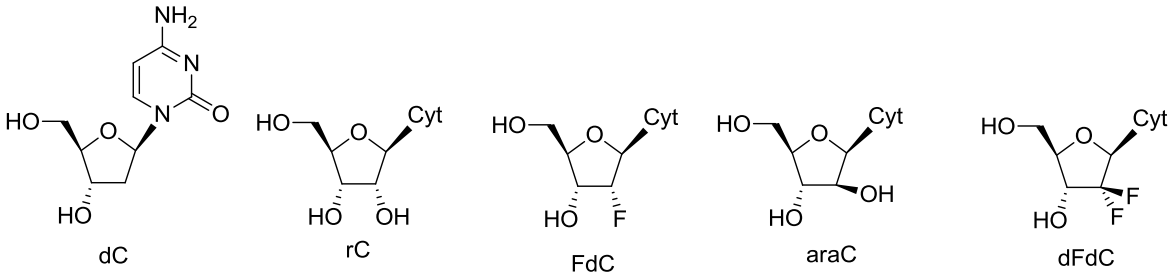

Figure 2. Structures of 2'-deoxycytidine (dC), 1-β-D-arabinofuranosylcytosine (araC), cytidine (rC), 2'-deoxy-2'-fluorocytidine (FdC), and 2'-deoxy-2'-2'-difluorocytidine (dFdC).

The incorporation of dCTP, rCTP, araCTP, FdCTP, and dFdCTP into the two single nucleotide gapped substrates DNA-1 and DNA-3 pol β was examined under steady-state conditions. The results are summarized in Figure 4A. In both sequences, the k_{cat}/K_m for rCTP was three-orders of magnitude less than that of dCTP, similar to previous results. The k_{cat}/K_m for araCTP and dFdCTP was 5 to 40-fold less than dCTP in DNA-1 but 1200 to 2400-fold less with DNA-3. The kinetics of incorporation of FdCTP also depended on sequence. In the DNA-1 context, FdCTP was incorporated as rapidly as araCTP and dFdCTP while with DNA-3, the rate was as slow as rCTP. To gain a greater understanding of the mechanism, we evaluated the reaction under single-turnover conditions.

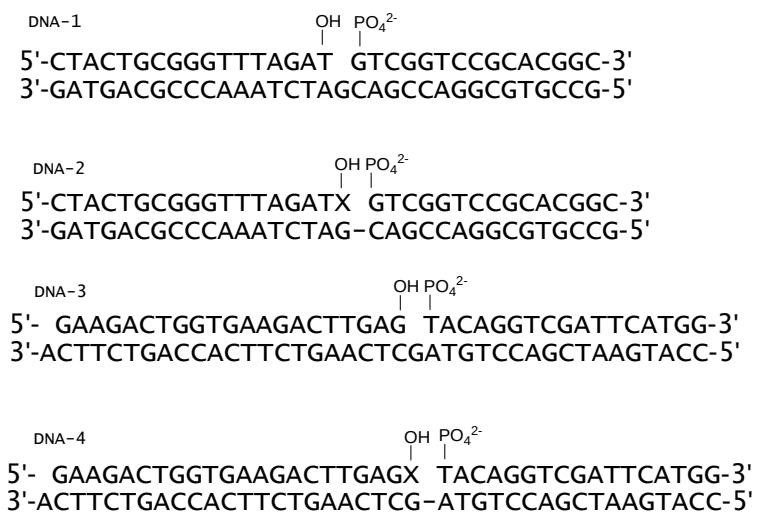

Figure 3 Sequences of the DNA substrates. DNA-1 and DNA-3 have a single nucleotide gapped used for DNA polymerase beta experiments. DNA-2 and DNA-4 are nicked DNA duplexes used for the DNA ligase experiments. The identity of X is dC, rC, araC, dFdC, or FdC.

The pre-steady-state kinetics parameters we determined by following the time course of reaction with 25 nM DNA, 100 nM polymerase and various concentrations of triphosphate. The resulting first-order rate constants were plotted against triphosphate concentrations to obtain the k_{pol} and K_d^{dNTP} parameters. As found previously, the rate of incorporation of rC is reduced due to a decrease in the k_{pol}, and not the K_d parameter.

In contrast to the steady-state results, the rate of incorporation of dFdC and araC were very similar to that of dCTP. This result indicates that polymerase beta can incorporate dFdC opposite dG very efficiently. The reduced k_{cat}/K_m parameters may be due to reduced dissociation of the DNA from the polymerase.

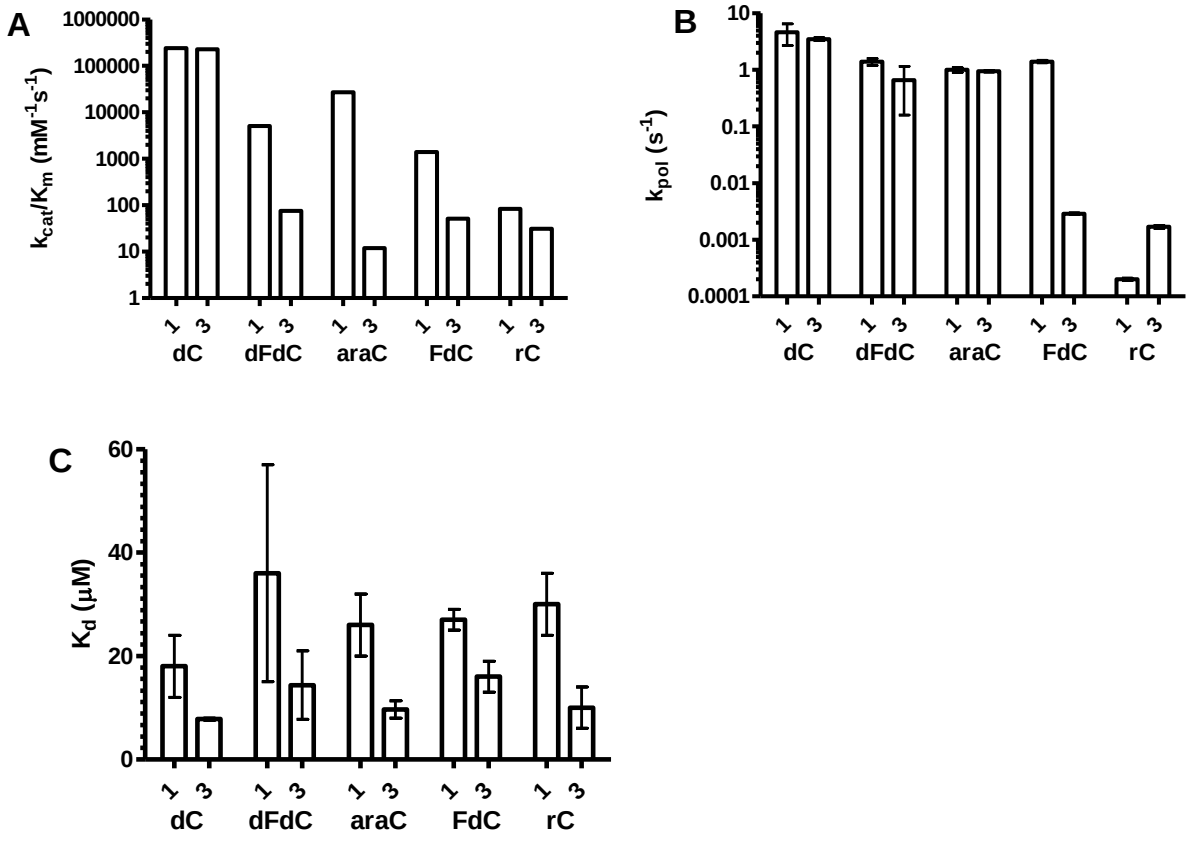

Figure 4. Kinetic parameters for the incorporation of dC and analogs opposite dG. A. Steady-state kinetic parameters. B The pre-steady-state parameter k_{pol}. C. The affinity of the dNTP to the DNA-polymerase complex. On the x-axis of each graph is an indication of the DNAsubstrate used, either DNA-1 or DNA-3, and the identity of the dNTP. The error bars represent the standard deviation of at least three measurements.

Reactivity of DNA ligase with DNA containing dFdC.

The nicked substrates for the ligation experiments were synthesized *in situ* by incubating pol β and NTP with the single nucleotide gapped substrates DNA-1 and DNA-3 to produce DNA-2 and DNA-4. The ability of the products of the pol β reactions to be ligated was initially evaluated with T4 ligase. The ligation was monitored by PAGE. The ligation was performed using enzyme in excess over DNA (40 nM T4 ligase and 10 nM DNA) and was analyzed by first order kinetics. The first-order rate constants are presented in Figure 5A and B. Ligation was rapid for DNA-2 but was 50-fold slower for DNA-4. The identity of the nucleotide does not significantly influence the reaction rate. The maximum rate reduction was 5-fold with dFdC in DNA-2. Previously, it was found that araC was ligated approximately 3-fold more slowly than dC when placed at the 3′-terminus. We found that araC affected the rate of incorporation minimally.

To gain a better understanding of what may be occurring in humans, we examined the ability of the ligase III/XRCC1 complex to seal the nicked DNA substrates. Ligation was performed as described above with a four-fold excess of ligase III/XRCC1 over DNA. The results show that the rate of ligation with dC by ligase III/XRCC1 was less affected by DNA sequence than T4 ligase; DNA-2 was ligated 4-fold faster than DNA-4. As with T4 ligase, the identity of the nucleotide at the 3′-terminus did not significantly affect the rate of ligation. In DNA-2, the rate of ligation with dFdC was reduced 10-fold and the rate of ligation with araC was reduced 60-fold. However in DNA-4, dFdC did not affect the rate of ligation while araC slowed down ligation only 3-fold.

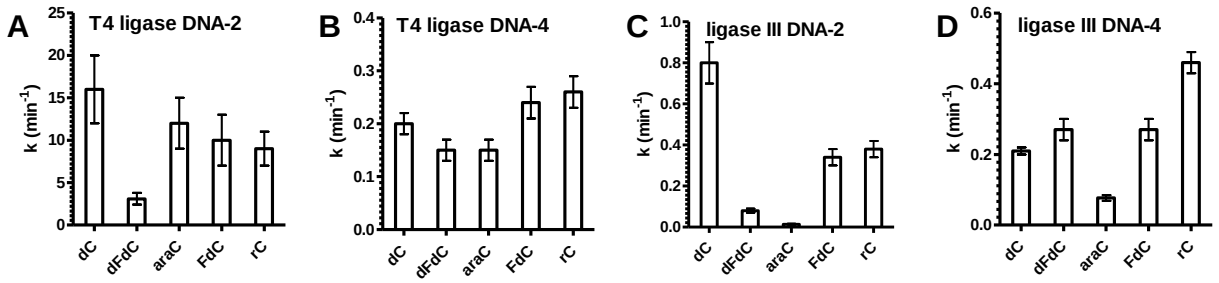

Figure 5. Rate of ligation of DNA-2 and DNA-4 catalyzed by T4 ligase and ligase III/XRCC1.

Conclusions.

Figure 6 summarizes the results as to whether dFdC can be incorporated into the DNA via BER. The data suggests that dFdC can be incorporated into the DNA, but a reduced rate when compared with dCTP. The rate constants are the average of the two DNA sequences that we studied. The binding affinity of dFdCTP is half that of dCTP, while the rate of phosphodiester bond formation is ¼ that of dCTP. The rate of ligation is also slowed.

Our results indicate that pol β has considerable opportunity to incorporate dFdC and araC into DNA. DNA ligase III/XRCC1 can readily ligate the DNA containing dFdC thereby effectively incorporating dFdC into the DNA. dFdC can then cause toxicity by inhibiting DNA replication during S-phase of the cell cycle. In contrast, we would not expect araC to be ligated by ligase III/XRCC1 resulting in single-strand that would also be toxic to the cells during S-phase.

Several tumor tissues have been shown to have increased expression of pol β, which may provide more opportunity for the incorporation of

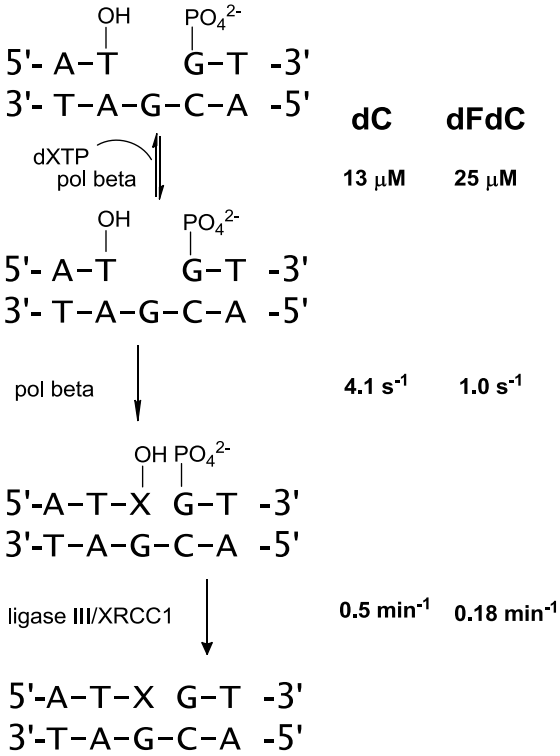

Figure 6. Summary of the rates of incorporation of dFdC into the DNA and ligation

gemcitabine into the genome via the BER pathway and thus make it more cytotoxic in individuals with these types of tumors.

2. Correlation of Gemcitabine DNA and DNA polymerase levels.

Our overall hypothesis is that translesion DNA polymerases are able to incorporate dFdC into the DNA of cells thereby bypassing the inhibition on high fidelity DNA polymerases. While we did not obtain cells from patients, we are able to test our hypothesis on cultured cells.

The levels of gemcitabine (dFdC) incorporated into the DNA of six cell lines was determined. The cells were grown in the appropriate media and during cell growth, treated with a 10 μM dose of gemcitabine for 12 h. The cells were harvested, washed, and the DNA isolated. The DNA was enzymatically hydrolyzed and the sample was divided into 2 aliquots at a 9:1 ratio. The DNA concentration was measured on the smaller aliquot using HPLC with UV detection. The remainder of the sample was used for dFdC determination via the HPLC-MS/MS method using the MDS/Sciex 4000 QTrap in the core facility. The experiment was repeated three times. The data in Figure 7 show that the amount of dFdC incorporated into the DNA is dependent on the cell line.

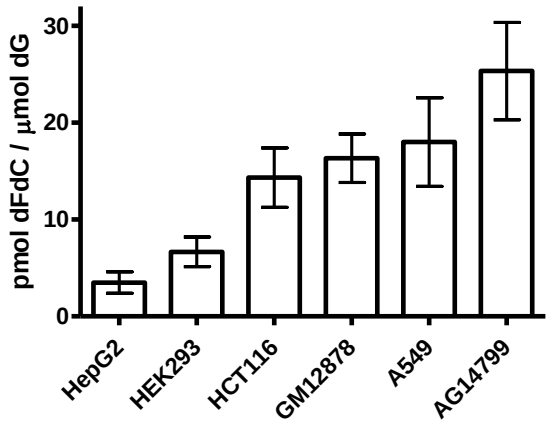

Figure 7. Incorporation of dFdC into the DNA of cells. The value represents the mean ± standard deviation of three separate experiments.

To test the hypothesis that the levels of DNA polymerases affect the incorporation of the dFdC, we measured the levels of six DNA polymerases by Western analysis. The antibodies for pol beta, delta, iota, and zeta were purchased from Santa Cruz and those for eta and kappa were purchased from Abcam. GAPDH was used as a control.

Cells were isolated and the membrane lysed in the presence of protease inhibitors. The protein concentration was determined by the biuret assay and equal amounts of protein were applied to SDS PAGE. The material was transferred from the gel to a membrane and the different primary and secondary (goat anti-human IgG bound to horse radish peroxidase) antibodies were applied to analyze the relative amounts of the various proteins. The amounts of proteins were measured via chemiluminensence. Figure 8 shows the levels of protein with respect to GAPDH. For each polymerase, a sample from the HepG2 cell line was set at 100. The experiment was repeated on three consecutive days from the same stock cell lines. The data plotted are the mean ± standard deviation. The data show that the levels of polymerases vary up to five fold in our samples.

To determine if the level of any polymerase correlated with gemcitabine incorporation, the levels of protein were plotted against gemcitabine incorporation as shown in Figure 9. The data were analyzed by linear regression and the best fit line and 95% confidence limits plotted. The slopes with the standard errors and the P-value for testing whether the slope is different from zero are shown in Table 1. The P values are not corrected for multiple comparisons.

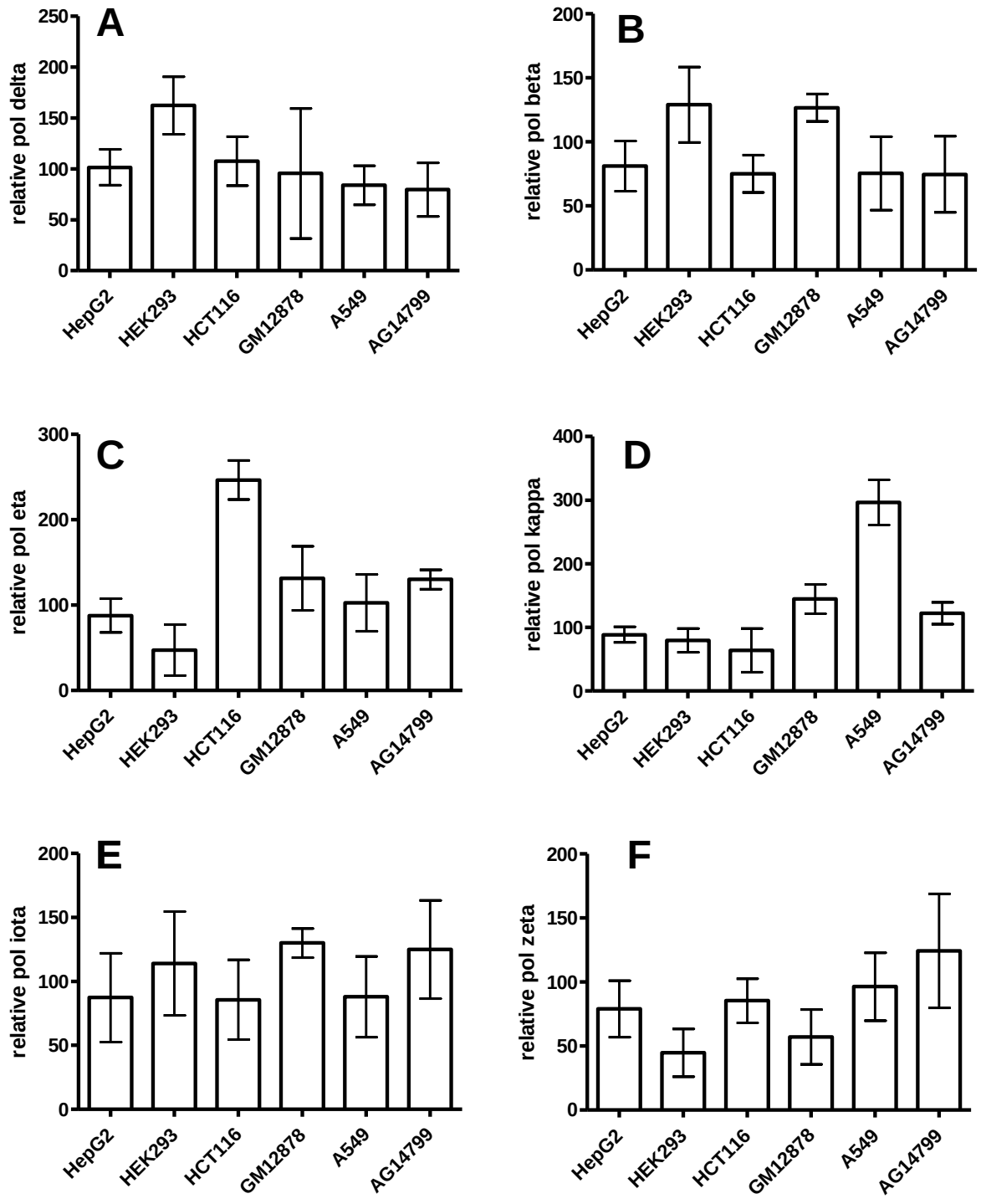

Figure 8. Relative levels of polymerases in different cell lines. A, pol δ; B, pol β; C, pol η; D, pol κ; E pol ι; F, pol ζ. The cells were isolate three times on separate days. The error bars are the standard deviations.

If a polymerase was responsible for the incorporation of dFdC into the DNA, then we would expect to observe a positive correlation between the amount of the polymerase and level of dFdC in the DNA. The most positive results are with DNA polymerase zeta in which we observe a slope of 2.5 ± 1.3. However, there is such variability in the data that the P value for a difference of the slope from zero was 0.12 and consequently did not reach statistical significance. Biochemically, this result makes the most sense as DNA polymerase zeta has been found to be effective in extending mispairs and DNA damage. This is precisely the step where dFdC has been found to inhibit DNA synthesis.

Table 1. Slope and P value of line in Figure 9.

Polymerase	Slope ± SE	P value (uncorrected)
Pol delta	-2.4 ± 1.4	0.16
Pol beta	-1.1 ± 1.6	0.52
Pol eta	3.1 ± 4.0	0.47
Pol kappa	4.4 ± 4.9	0.42
Pol zeta	2.5 ± 1.3	0.12
Pol iota	1.1 ± 1.2	0.42

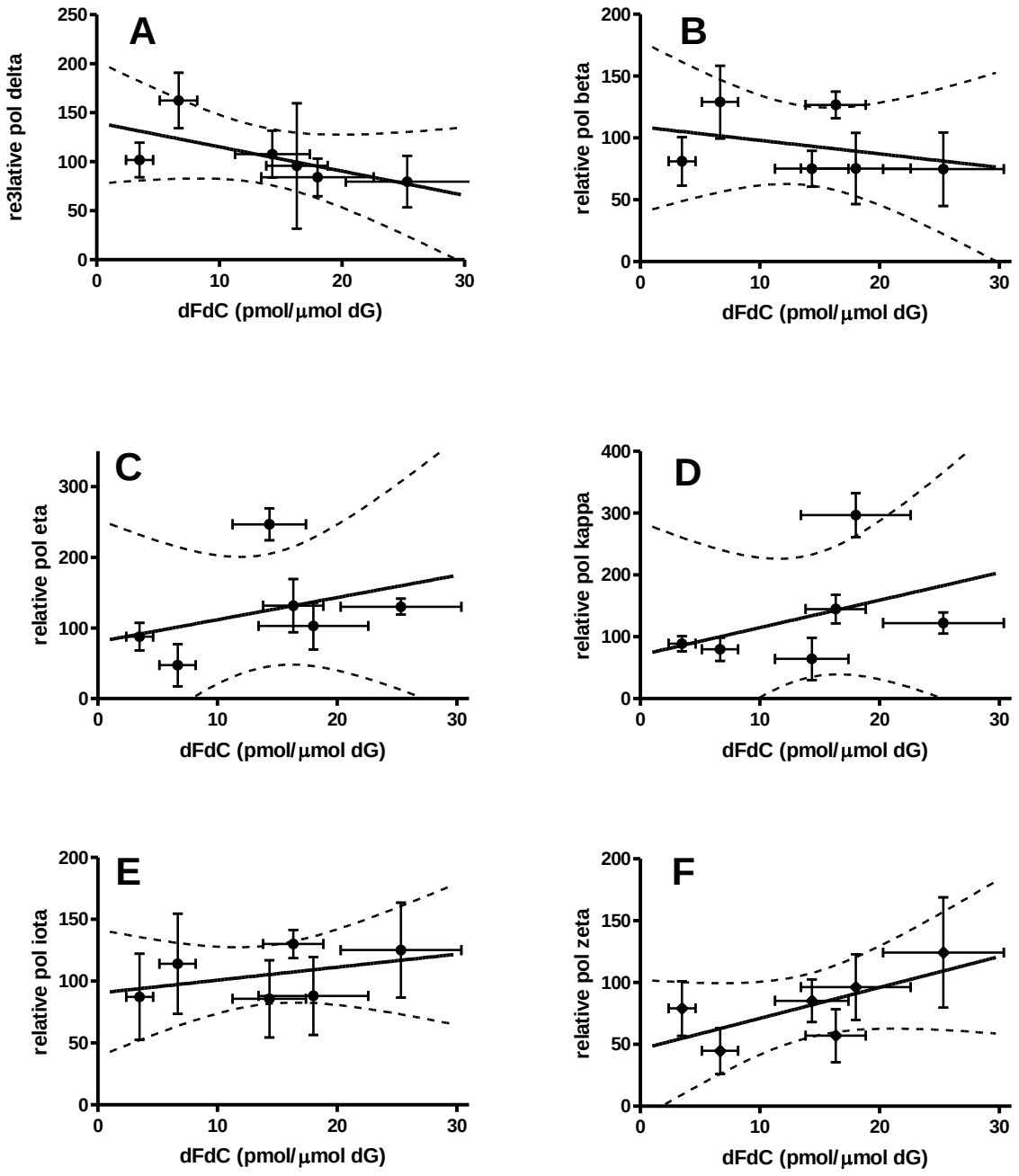

Figure 9. Plot of relative polymerase level versus dFdC incorporation. The error bars show the standard deviation of three experiments. The solid line is the least-squares best fit, and the dotted lines show the 95% confidence interval of the line.

18. Extent of Clinical Activities Initiated and Completed. Items 18(A) and 18(B) should be completed for all research projects. If the project was restricted to secondary analysis of clinical data or data analysis of clinical research, then responses to 18(A) and 18(B) should be "No."

18(A) Did you initiate a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_X___Yes
_____No

18(B) Did you complete a study that involved the testing of treatment, prevention or diagnostic procedures on human subjects?

_____Yes
__X___No

If "Yes" to either 18(A) or 18(B), items 18(C) – (F) must also be completed. (Do NOT complete 18(C-F) if 18(A) and 18(B) are both "No.")

18(C) How many hospital and health care professionals were involved in the research project?

__1___Number of hospital and health care professionals involved in the research project

18(D) How many subjects were included in the study compared to targeted goals?

__50___Number of subjects originally targeted to be included in the study
___1___Number of subjects enrolled in the study

Note: Studies that fall dramatically short on recruitment are encouraged to provide the details of their recruitment efforts in Item 17, Progress in Achieving Research Goals, Objectives and Aims. For example, the number of eligible subjects approached, the number that refused to participate and the reasons for refusal. Without this information it is difficult to discern whether eligibility criteria were too restrictive or the study simply did not appeal to subjects.

18(E) How many subjects were enrolled in the study by gender, ethnicity and race?

Gender:
__1___Males
_____Females
_____Unknown

Ethnicity:
_____Latinos or Hispanics
__1___Not Latinos or Hispanics
_____Unknown

Race:
_____American Indian or Alaska Native
_____Asian
_____Blacks or African American
_____Native Hawaiian or Other Pacific Islander
_1____White
_____Other, specify:_____
_____Unknown

18(F) Where was the research study conducted? (List the county where the research study was conducted. If the treatment, prevention and diagnostic tests were offered in more than one county, list all of the counties where the research study was conducted.)

Dauphin

19. Human Embryonic Stem Cell Research. Item 19(A) should be completed for all research projects. If the research project involved human embryonic stem cells, items 19(B) and 19(C) must also be completed.

19(A) Did this project involve, in any capacity, human embryonic stem cells?
_____Yes
_X___ No

19(B) Were these stem cell lines NIH-approved lines that were derived outside of Pennsylvania?
_____Yes
_____ No

19(C) Please describe how this project involved human embryonic stem cells:

20. Articles Submitted to Peer-Reviewed Publications.

20(A) Identify all publications that resulted from the research performed during the funding period and that have been submitted to peer-reviewed publications. Do not list journal abstracts or presentations at professional meetings; abstract and meeting presentations should be listed at the end of item 17. **Include only those publications that acknowledge the Pennsylvania Department of Health as a funding source** (as required in the grant agreement). List the title of the journal article, the authors, the name of the peer-reviewed publication, the month and year when it was submitted, and the status of publication (submitted for publication, accepted for publication or published.). Submit an electronic copy of each publication or paper submitted for publication, listed in the table, in a PDF version 5.0.5 (or greater) format, 1,200 dpi. Filenames for each publication should include the number of the research project, the last name of the PI, the number of the publication and

an abbreviated research project title. For example, if you submit two publications for PI Smith for the "Cognition and MRI in Older Adults" research project (Project 1), and two publications for PI Zhang for the "Lung Cancer" research project (Project 3), the filenames should be:

Project 1 – Smith – Publication 1 – Cognition and MRI
Project 1 – Smith – Publication 2 – Cognition and MRI
Project 3 – Zhang – Publication 1 – Lung Cancer
Project 3 – Zhang – Publication 2 – Lung Cancer

If the publication is not available electronically, provide 5 paper copies of the publication.

Note: The grant agreement requires that recipients acknowledge the Pennsylvania Department of Health funding in all publications. Please ensure that all publications listed acknowledge the Department of Health funding. If a publication does not acknowledge the funding from the Commonwealth, do not list the publication.

Title of Journal Article:	Authors:	Name of Peer-reviewed Publication:	Month and Year Submitted:	Publication Status (check appropriate box below):
1. Incorporation of gemcitabine and cytarabine into DNA by DNA polymerase beta and ligase III/XRCC1	Prakasha Gowda AS, Polizzi JM, Eckert KA, Spratt TE.	Biochemistry	February 2010	☐Submitted ☐Accepted ☑Published

20(B) Based on this project, are you planning to submit articles to peer-reviewed publications in the future?

Yes_____ No___X_____

If yes, please describe your plans:

21. Changes in Outcome, Impact and Effectiveness Attributable to the Research Project. Describe the outcome, impact, and effectiveness of the research project by summarizing its impact on the incidence of disease, death from disease, stage of disease at time of diagnosis, or other relevant measures of outcome, impact or effectiveness of the research project. If there were no changes, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

22. Major Discoveries, New Drugs, and New Approaches for Prevention Diagnosis and Treatment. Describe major discoveries, new drugs, and new approaches for prevention,

diagnosis and treatment that are attributable to the completed research project. If there were no major discoveries, drugs or approaches, insert "None"; do not use "Not applicable." Responses must be single-spaced below, and no smaller than 12-point type. DO NOT DELETE THESE INSTRUCTIONS. There is no limit to the length of your response.

None

23. Inventions, Patents and Commercial Development Opportunities.

23(A) Were any inventions, which may be patentable or otherwise protectable under Title 35 of the United States Code, conceived or first actually reduced to practice in the performance of work under this health research grant? Yes_____ No____X_____

If "Yes" to 23(A), complete items a – g below for each invention. (Do NOT complete items a - g if 23(A) is "No.")

a. Title of Invention:

b. Name of Inventor(s):

c. Technical Description of Invention (describe nature, purpose, operation and physical, chemical, biological or electrical characteristics of the invention):

d. Was a patent filed for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____

If yes, indicate date patent was filed:

e. Was a patent issued for the invention conceived or first actually reduced to practice in the performance of work under this health research grant?
Yes_____ No____
If yes, indicate number of patent, title and date issued:
Patent number:
Title of patent:
Date issued:

f. Were any licenses granted for the patent obtained as a result of work performed under this health research grant? Yes_____ No____

If yes, how many licenses were granted?_____

g. Were any commercial development activities taken to develop the invention into a commercial product or service for manufacture or sale? Yes___ No____

If yes, describe the commercial development activities:

23(B) Based on the results of this project, are you planning to file for any licenses or patents, or undertake any commercial development opportunities in the future?

Yes_____ No____X_____

If yes, please describe your plans:

24. Key Investigator Qualifications. Briefly describe the education, research interests and experience and professional commitments of the Principal Investigator and all other key investigators. In place of narrative you may insert the NIH biosketch form here; however, please limit each biosketch to 1-2 pages. *For Nonformula grants only – include information for only those key investigators whose biosketches were not included in the original grant application.*

Thomas E. Spratt, PhD

INSTITUTION AND LOCATION	DEGREE *(if applicable)*	YEAR(s)	FIELD OF STUDY
University of Rochester, Rochester NY	BA	1978	Chemistry
University of Chicago, Chicago IL	PhD	1985	Bioorganic chemistry
Ohio State University, Columbus OH	Post-PhD	1986	Bioorganic chemistry
American Health Foundation Valhalla NY	Post-PhD	1988	Carcinogenesis

Positions

American Health Foundation, Valhalla, NY

1988-2004	Associate Research Scientist(1988-1994), Research Scientist (1994-2000), Scientist (2000-2004), Division of Chemical Carcinogenesis,
2004	Leader of Mechanisms of Carcinogenesis program of the American Health Foundation Cancer Center Purchase College, State University of New York
1999	Assistant Professor, teachibng one semester of Organic Chemistry. Pennsylvania State University
2004 – present	Associate Professor of Biochemistry and Molecular Biology. Pennsylvania State University College of Medicine

Honors

2004-2007	Editorial Advisory Board of Chemical Research in Toxicology
2006-present	Chair, Publicity Committee, Chemical Toxicology Division of the ACS
2007-2011	Elected Secretary, Chemical Toxicology Division of the ACS