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ADDENDUM SHEET FOR
WORK UNIT M4305.05-3089AGG6

1. Title: (U) Cytosine arabinoside treatment of smallpox (variola minor: effects on the virus and the disease

2. Planning Estimates	FY74
A. Man Years (Total	1.3
1. Military	0.3
Professional	0.3
Supporting	-
2. Civilian	1.1
Professional	0.1
Supporting	1.0
B. Funds (Total)	3.5
1. BUMED Research	3.5
Personal Services	2.5
Material	1.0
Other	-
2. Contributing	-

C. Travel: N/A

D. Contracts: N/A

E. Facilities: N/A

F. Equipment: N/A

G. Continuing Costs: N/A

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3. Background and Objectives:

In spite of massive efforts at control, smallpox remains endemic in several areas of the world (notably Ethiopia, Sudan, the Indian sub-continent, and Indonesia); approximately 50,000 cases were reported to WHO for 1971 (1). Unvaccinated persons of all races and ages are susceptible to smallpox, and in an age of rapid international travel the potential for limited but disastrous outbreaks exists worldwide. This is exemplified by the present outbreaks in Yugoslavia, Syria, and Bangla Desh. Recently, the Surgeon General of the U.S. Public Health Service recommended that routine smallpox vaccination be discontinued in the United States (2) and also advised that vaccination for travel in Europe and the U.S.S.R. was no longer required. The subsequent precipitous fall in the amount of vaccine distributed (3) underlines the importance of a continued search for an effective method of treatment of the disease until such time that worldwide eradication has been achieved.

Although administration of vaccinial immune globulin (4) and thiosemicarbazones (N-methylisatin-beta-thiosemicarbazone) appear to afford some protection in smallpox contacts, there is presently no effective treatment of the disease. Reports suggesting that smallpox responds to treatment with 5-iodouracil-2'-deoxyriboside (IUdR) have not been substantiated.

Cytosine arabinoside (1-beta-D-arabinofuranosyl cytosine), a synthetic pyrimidine nucleoside, is a DNA antagonist widely investigated for its anti-tumor properties. Studies have, moreover, shown the drug to possess a potent *in vitro* and *in vivo* antiviral activity (6). It exerts its greatest effect in tissue culture against the herpes virus (7, 8), cytomegalic virus (9), and vaccinia (10). The drug has been shown effective in the treatment of varicella (11, 12), herpes (13, 14, 15, 16) and cytomegalic (17) virus disease in humans.

Smallpox virus (*Poxvirus variolae*) contains a DNA core and its replication is therefore subject to inhibition by substances that interfere with DNA synthesis. On the basis of detailed biochemical studies (18) and on its observed *in vitro* and *in vivo* effects against DNA viruses, it is logical that a study be made of the therapeutic value of cytosine arabinoside in smallpox patients.

Smallpox disease (variola minor) is endemic in Ethiopia, with greater than 25,000 cases reported to WHO for 1971. Isolation of small numbers of referred patients at the Ministry of Health's Anti-Epidemic Service Hospital in Addis Ababa provides the opportunity for clinical investigation of this disease by NAMRU-3 Research Detachment staff.

The informational data for clinical investigators intending to use this drug have been carefully reviewed. Liaison has been established with officials representing the participating host country

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organizations and institutions (Ministry of Public Health Smallpox Eradication Programme and Anti-Epidemic Service Hospital). The protocol has been reviewed and tentatively approved by the sponsor (George L. Royer, Jr., M.D., The Upjohn Company, Kalamazoo, Michigan) and by Dr. G.E. Underwood (Virology Department, The Upjohn Company).

4. Technical Approach:

a. Patient population: Thirty patients with acute smallpox will be chosen for study from admissions to the Anti-Epidemic Service Hospital, Addis Ababa. They will be between the ages of 3-50 years; will not be critically ill, and will be in the early stages (macular, papular, vesicular, or pustular) of their illness; will not have concomitant bacterial infection or other compromising disease; will have pre-treatment WBC counts of 4,000/mm^3 or greater, hemoglobin levels of 10 gm% or greater, and platelet counts of 100,000/mm^3 or above; and will give informed consent for participation. Although an attempt will be made to obtain written consent, this may prove impossible in the majority of cases. Ethiopians are reluctant to affix their signatures to any document and generally do not understand scientific explanations of the cause and treatment of infectious diseases. Insistence on written consent may well jeopardize the success of the study. In those patients from whom written consent is not obtained, oral consent will be obtained and the fact recorded on the patients' charts.

b. History and physical: Each patient will have a complete history and physical examination performed by a NAMRU-3 physician prior to treatment. A standardized checklist will be used for daily notation of pertinent symptoms and signs during the period of hospitalization.

c. Clinical laboratory studies: Each patient will have a CBC, platelet count, blood culture, blood smear for parasites, and complete urine analysis performed on admission. CBCs and platelet counts will be performed daily during therapy and then twice weekly during the 2nd and 3rd post-treatment weeks. Serum will be collected for blood urea nitrogen, creatinine, serum glutamic oxalacetic transaminase, bilirubin and protein determinations at time of admission and then once weekly after initiation of treatment.

d. Virologic studies: Serum for antibody determinations will be collected prior to treatment and twice weekly during hospitalization. Scrapings from macular or

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papular lesions, fluid from vesicals, pus, or crusts will be collected daily for the first 7 days of hospitalization and twice weekly thereafter. Specimens will be stored at -70°C until transported in liquid nitrogen to Dr. Harold E. Renis, Pathogenic Virus Laboratory, Upjohn Company, Kalamazoo, Michigan.

e. Treatment schedule: Cytosine arabinoside will be administered in a double-blind fashion with blinded drug and placebo provided by the Upjohn Company, Kalamazoo, Michigan. Dosage will be 100 mg/sq m of calculated body surface per day by iv push for four (4) consecutive days. The code may be broken at any time at the discretion of the responsible physician. Therapy will be stopped if the platelet count falls below 75,000/mm^3 or if the WBC falls to less than 3,000 cells/mm^3.

f. Results: Drug effectiveness will be assessed on the basis of a comparison of clinical response, serologic, virologic, and general laboratory data between the control and treatment patient groups.

5. Publications:

1. Morbidity and Mortality Weekly Report, Center for Disease Control, Public Health Service, 21(3), January 28, 1972.

2. Morbidity and Mortality Weekly Report, Center for Disease Control, Public Health Service, 20(38), October 1, 1971.

3. Morbidity and Mortality Weekly Report, Center for Disease Control, Public Health Service, 21(13), April 7, 1972.

4. Kempe, H.C., Berge, T., and England, B.: Hyperimmune vaccine gamma globulin: Source, evaluation and use in prophylaxis and therapy. Pediatrics 18:177, 1956.

5. Bauer, D.J., St. Vincent, L., Kempe, C.H., and Downie, A.W.: Prophylactic treatment of smallpox contacts with N-methylisitan-beta-thiosemicarbazone (compound 33T56, Marbaron). Lancet 2:494, 1963.

6. Cohen, S.S.: Introduction to the biochemistry of D-arabinosyl nucleosides. Progr. Nucleic Acid Res. 5:1-88, 1966.

7. Buthala, D.A.: Cell culture studies on antiviral agents. I. Action of Ara-C and some comparisons with 5-iodo 2-desoxyuridine. Proc. Soc. Exp. Biol. Med. 115:69-77, 1964.

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8. Rapp, F.: Inhibition by metabolic analogues of plague formation by herpes zoster and herpes simplex viruses. J. Immun. 93:643-8, 1964.

9. Sedwell, R.W., et al. Bacteriol. Proc., pp 191, 1969.

10. Renis, H.E.: Comparison of cytotoxicity and antiviral activity of 1-beta-D-arabinofuranosyl-5-iodocytasine with related compounds. Cancer Res. 30:189-194, 1970.

11. Prager, D., Bruder, M., Sawitsky, A.: Disseminated varicella in a patient with acute myelogeneous leukemia: Treatment with cytosine arabinoside. J. Pediat. 78:321-323, 1971.

12. Juel-Jensen, B.E.: Varicella and cytosine arabinoside. Lancet 1:572, 1970.

13. McKelvey, E.M., Kwaan, H.C.: Cytosine arabinoside therapy for disseminated herpes zoster in a patient with IgG pyroglobulinemia. Blood 34:706-711, 1969.

14. Hall, T.C., et al: Treatment of varicella-zoster with cytosine arabinoside. Trans. Ass. Amer. Phys. 82:201-210, 1969.

15. Chow, A.W., Foerster, J., Hryniuk, W.: Cytosine arabinoside therapy for herpesvirus infections. Antimicrobial Agents and Chemotherapy, pp 214-217, 1970.

16. Hryniuk, W., Foerster, J., Shasania, M., and Chow, A.W.: Cytosine for herpes-virus infections. J.A.M.A. 219:715-718, 1972.

17. Plotkin, S.A., Stetler, H.: Treatment of congenital cytomegalic inclusion disease with antiviral agents. Antimicrobial Agents and Chemotherapy, pp 372-379, 1969.

18. Chu, M.Y., Fischer, G.A.: A proposed mechanism of action of 1-beta-D-arabinofuranosyl cytosine as an inhibitor of the growth of leukemic cells. Biochem. Pharmacol. 11:423-430, 1962.