Vaccine Trials for Osteogenic Sarcoma

A Preliminary Report*

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In June, 1963, a lyophilized homogeneous vaccine prepared from the primary tumor tissue was given to a child with osteogenic sarcoma and pulmonary metastases. During the 15 day course of injections the child gained seven pounds; following treatment the disease progressed and the patient died. However, the temporary improvement suggested that immunotherapy may have inhibited tumor growth. A formal controlled trial of immunotherapy in less advanced disease was begun in April, 1966. Patients under the age of 25 with osteogenic sarcoma of the long bones but no evidence of spread beyond the primary site and who had been treated by amputation were included in the study. The autogenous vaccine was given in the postoperative period following amputation. If metastases (usually pulmonary) appeared, the vaccine was judged a failure.

If there was subsequent pulmonary resection for metastases, any observed beneficial effect was credited to this surgical treatment. A preliminary report is presented on the patients who received vaccine treatment through March, 1972.

Diagnosis of Osteogenic Sarcoma

Several months of dull, low-grade pain and gradual swelling are the most common clinical signs of osteogenic sarcoma. If the pain is more intense or the swelling more sudden than expected, and if the radiologic diagnosis is equivocal, other possibilities, such as osteomyelitis, eosinophilic granuloma or aneurysmal bone cyst, should be considered.

The radiologic appearance of osteogenic sarcoma usually shows an osteolytic, osteoblastic or mixed picture. An adjacent soft tissue mass can often be identified with productive radiologic densities consistent with ossification, calcification or both. Skipped areas of tumor up and down the involved bone shaft are generally so small that they are not found on the initial X-ray. Medullary extension of the sarcoma is usually distal to the soft tissue mass.

The histopathology of osteogenic sarcoma is variegated. The essential finding, however, is a malignant spindle cell stroma forming osteoid or immature bone which is neither reactive nor periosteal. As the bone production becomes denser, the osteocytes become more mature (Phemister's normalization process). Large areas of spindle cells
or cartilage formation may be present, including bone formation due to endochondral ossification. This process of bone formation is also seen in chondrosarcoma and therefore is not in itself diagnostic for osteogenic sarcoma. Also, a small biopsy specimen, especially from a needle aspiration, may show only a few spindle cells which can be seen in reticulum cell sarcoma or fibrosarcoma, making a false diagnosis a real possibility. Likewise, a limited biopsy may show only cartilage, mimicking a diagnosis of a chondrosarcoma or giant cells, large angiomatous formations, mimicking a giant cell tumor or angiosarcoma. For these reasons, large, adequate, open biopsy specimens are necessary for accurate diagnosis.

**Materials and Methods**

A series of 145 consecutive patients with osteogenic sarcoma under the age of 21 treated between January 1949 and December 1965 has been previously reported in the literature. Nine patients, between the ages of 21 to 25, treated routinely (without vaccine) during this same period, were added to the present series (1966-1972) as a control group for the vaccine study. Data on patients who received vaccine treatment are summarized in Tables 1, and 2A and 2B.

| No. | Initials | Vaccine type | Sex | Age | Site of tumor | Pre-op radiation (1000 R) | Date amputation | Time from amp to met (mos) |
|-----|----------|--------------|-----|-----|---------------|--------------------------|-----------------|--------------------------|
| 1.  | PM       | whole cell   | F   | 15  | upper tibia   | 0                         | 5-1-68         | 20.0                     |
| 2.  | JS       | whole cell   | F   | 10  | upper femur   | 0                         | 5-16-68        | 16.1                     |
| 3.  | CP       | whole cell   | F   | 16  | upper humerus | 0                         | 7-22-68        | 7.0                      |
| 4.  | EM       | whole cell   | M   | 11  | upper tibia   | 0                         | 8-16-68        | 3.8                      |
| 5.  | KG       | whole cell   | F   | 12  | lower femur   | 0                         | 9-3-68         | 5.0                      |
| 6.  | SG       | whole cell   | F   | 10  | upper tibia   | 0                         | 10-2-68        | 11.2                     |
| 7.  | RB       | whole cell   | F   | 14  | upper fibula  | 0                         | 10-7-68        | 6.5                      |
| 8.  | DS       | whole cell   | M   | 16  | upper tibia   | 0                         | 10-30-68       | 4.8                      |
| 9.  | CR       | whole cell   | F   | 15  | lower femur   | 0                         | 12-10-68       | 4.2                      |
| 10. | EW       | whole cell   | M   | 19  | lower femur   | 0                         | 1-24-69        | 3.3                      |
| 11. | GP       | whole cell   | M   | 14  | lower femur   | 0                         | 8-22-69        | 4.3                      |
| 12. | MB       | whole cell   | F   | 20  | lower femur   | 0                         | 9-23-68        | 5.4                      |

| No. | Initials | Vaccine type | Sex | Age | Site of tumor | Pre-op radiation (1000 R) | Date amputation | Length of follow-up (mos) |
|-----|----------|--------------|-----|-----|---------------|--------------------------|-----------------|---------------------------|
| 13. | KK       | whole cell   | F   | 11  | upper tibia   | 0                         | 10-4-68         | 37.0                     |
Preparation of the Lysed Cell Vaccine

The trial began with the administration of a UV-irradiated lysed cell vaccine. This vaccine was prepared by putting the tumor specimen and approximately four times its weight of saline into a Virtilis homogenizer in an ice water bath, operated at maximum speed for 15 minutes. This procedure ruptured virtually all of the cells and a large proportion of the nuclei. The resulting suspension was then centrifuged for 15 minutes at 8,000 G which sedimented intact cells and nuclei but left the cell sap, microsomes, mitochondria and membranes in the harvested supernatant. Transmission of 253.7 m\(\mu\) ultraviolet light through this supernatant was measured in a Beckman DU Photometer; the specimen was diluted in sufficient isotonic saline to allow at least 50 percent transmission through a depth of 2 mm. If the preparation contained large amounts of hemoglobin, a considerable dilution was necessary to accomplish this objective. The preparation was then placed in open Petri dishes to a depth of 2 mm. and irradiated from a "germicidal" UV lamp to provide a dose of 50,000 ergs./mm.\(^2\) at the bottom of the fluid layer. The material was ampuleated aseptically in volumes of from 1 to 5 ml. Concentrations of these extracts and the total dosage given to the patients varied widely. We have no data on actual concentration or the amount of tissue solids which were administered but the irradiated supernatants represented the yield from as much as 200 mg. of tumor tissue per ml. to as little as 31 mg. per ml. The total dosage administered per patient was the yield from as much as 14 gm. of tumor tissue to as little as 1.8 gm. Vials were stored in a "Revco" freezer at approximately -60°C.

Preparation of the Whole Cell Vaccine

A gamma-irradiated whole cell suspension was prepared by mincing the tumor with scissors and scalpels; fragments were eliminated by sedimentation or by filtration through surgical gauze. The resulting suspension, which consisted predominantly of single cells, was

| No. | Initials | Vaccine Type | Sex | Age | Site of tumor | Pre-op radiation (1000 R) | Date of amputation | Time from amp to met (mos) |
|-----|----------|--------------|-----|-----|---------------|--------------------------|--------------------|---------------------------|
| 1   | JR       | lysed cell   | F   | 15  | left femur    | 0                        | 4-27-66            | 22.2                      |
| 2   | JH       | lysed cell   | M   | 17  | upper tibia   | 12                       | 7-1-66             | 7.7                       |
| 3   | GK       | lysed cell   | M   | 15  | upper tibia   | 12                       | 10-10-66           | 5.7                       |
| 4   | KW       | lysed cell   | M   | 8   | lower femur   | 12                       | 2-9-67             | 4.9                       |
| 5   | LJ       | lysed cell   | F   | 10  | upper humerus | 0                        | 2-23-67            | 10.3                      |
| 6   | RF       | lysed cell   | M   | 16  | upper tibia   | 0                        | 8-9-67             | 20.0                      |
| 7   | MC       | lysed cell   | M   | 11  | lower femur   | 0                        | 12-13-67           | 5.5                       |
| 8   | NH       | lysed cell   | F   | 16  | upper humerus | 4                        | 12-19-67           | 7.2                       |
| 9   | RS       | H\(_2\)O lysed | M   | 12  | upper humerus | 0                        | 9-5-69              | 4.5                       |
| 10  | JJ       | lysed cell   | M   | 16  | upper humerus | 0                        | 1-29-71            | 14.9                      |
| 11  | MC       | lysed cell   | F   | 9   | upper tibia   | 0                        | 9-15-71            | 5.7                       |
| 12  | AC       | lysed cell   | M   | 18  | upper humerus | 0                        | 11-3-71            | 4.3                       |
then centrifuged and the cells were re-suspended in Solution A (15) at a concentration of 10 million cells/ml. The suspensions were irradiated from a Cobalt 60 source to a dose of 10,000 rads, ampules in 1 ml aliquots and stored at -60°C. Immediately before administration, a vial of vaccine was allowed to thaw at room temperature. The contents were mixed by twice drawing a syringe in and out of the vial and then was administered by subcutaneous or intramuscular injection into the gluteal or deltoid region.

The injections were usually started between seven and 10 days after surgery (never later than 28 days) and continued at approximately biweekly intervals for as many doses as were available. Patients who lived outside the commuting area of this hospital received at least the first dose of vaccine while hospitalized and thereafter through the cooperation of their local physicians who received shipments of the vaccine packed in dry ice and stored it in a household-type deep freezer at temperatures around -10°C.

The gamma-irradiated whole cell suspension was given to patients during 1968 and 1969. Of the 13 patients who received the whole cell preparation, listed in Table 1, only one is still free of disease at this time. The whole cell vaccine has been abandoned as ineffective. The lysed cell series includes, however, three patients who received an H2O lysed cell preparation in 1969. Table 2A lists the patients in the lysed cell series who had a subsequent diagnosis of pulmonary metastasis and Table 2B lists those nine patients who have not developed pulmonary metastases to date.

**Results**

The race, sex, age, site of tumor, preoperative duration of symptoms and/or signs, amount of preoperative radiation (if given) and the date of amputation were recorded for each patient in the control and vaccine groups (whole cell and lysed cell vaccines). The date of latest follow-up was noted for patients with no evidence of disease; the time from amputation to last negative and first pos-

| No. | Initials | Vaccine type | Sex | Age | Site of tumor | Pre-op radiation (1000 R) | Date of amputation | Length of follow-up (mos) |
|-----|----------|--------------|-----|-----|---------------|---------------------------|--------------------|--------------------------|
| 13  | KP       | lysed cell   | F   | 4   | upper tibia   | 12                        | 5-16-66            | 69.1                     |
| 14  | CN       | lysed cell   | F   | 18  | upper tibia   | 12                        | 6-3-66             | 69.9                     |
| 15  | JP       | lysed cell   | M   | 15  | upper tibia   | 0                         | 4-10-67            | 56.4                     |
| 16  | PD       | lysed cell   | M   | 7   | upper humerus | 0                         | 7-20-67            | 56.1                     |
| 17  | WM       | H2O lysed    | M   | 24  | lower femur   | 0                         | 2-14-69            | 32.4                     |
| 18  | JC       | H2O lysed    | M   | 20  | lower femur   | 0                         | 3-3-69             | 38.6                     |
| 19  | RB       | lysed cell   | F   | 17  | upper tibia   | 0                         | 11-9-71            | 5.1                      |
| 20  | CK       | lysed cell   | M   | 23  | lower femur   | 0                         | 1-13-72            | 2.3                      |
| 21  | EV       | lysed cell   | M   | 16  | upper humerus | 0                         | 2-17-72            | 1.4                      |
itive chest X-ray was noted for those patients with pulmonary metastases. If the chest X-ray was positive for metastases, the vaccine was judged a failure in that patient.

An historical control, including patients treated before the vaccine trials were begun, was employed as the scarcity of patients with osteogenic sarcoma did not permit randomization. However, the control group consisted of a consecutive series of cases from this hospital and all slides were reviewed by the same pathologist.

The distribution of patients by race, sex, age, site and preoperative duration was similar in the control and the vaccine series. Some patients in the control and lysed cell series had received preoperative radiation therapy, while none in the whole cell series was irradiated prior to surgery. However, analysis of the control series showed that preoperative radiation therapy had no effect on the course of the disease.\textsuperscript{10}

The length of the disease-free interval from surgery (surgery entailed amputation except in one case) to the onset of demonstrable pulmonary metastasis (the date of the first positive X-ray) was felt to be the most suitable criterion for evaluating the effect of the vaccine. It is important to note, however, that X-rays were not taken on as regular a basis in the control series as in the vaccine series. Thus, metastases tended to be diagnosed at a later date, making the disease-free interval in the control series appear longer. The median interval from the last negative to the first positive chest film was seven months for the control series and two months for the vaccine series. For 28 patients in the control series the closest available date for the onset of pulmonary metastases was the date of death, because of the complete lack of follow-up X-ray examination.

A comparison of the disease-free intervals for the three groups (control, whole cell and lysed cell), computed by means of the life table method.\textsuperscript{11} is shown in the Figure. The relationship between two such curves may be evaluated by the Wilcoxon-Gehan test.\textsuperscript{12} On this basis, there is no statistically significant difference between the control series and either vaccine series. However, comparison of the two vaccine groups yields $P < .02$ (two-sided test).

Taking into consideration the lack of regular follow-up for patients in the control series (which causes the control series curve to be higher than if pulmonary metastases had been diagnosed more promptly), it might be conjectured that the whole cell vaccine group is perhaps similar in effect to the control, that is, the whole cell vaccine is ineffective and that the lysed cell vaccine group performs more successfully in relation to the control than appears here. When comparing these two groups, $P < .16$ was obtained or, equivalently, $P < .08$ if a one-sided test is considered.

As mentioned, use of the whole cell vaccine has been abandoned. Continuation of the trial using the lysed cell preparation is indicated, however, be-
cause of the strong favorable trend evident in our results to date.

**Summary**

Twenty-one patients with osteogenic sarcoma of the long bones treated by a lysed cell vaccine, in addition to routine amputation, appear to have an encouraging response to vaccine treatment, compared with both the whole cell vaccine and the control series. (See Figure below.)

In view of the infrequency of follow-up chest films in the control group, an inherent bias is present in our data, making the time to metastasis for control pa-

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**OSTEOGENIC SARCOMA**

|                      | CONTROL SERIES: 154 patients |
|----------------------|------------------------------|
|                      | (27 alive and well)          |
| LYSED CELL VACCINE  | 21 patients (9 alive and well; lengths of follow-up indicated by ◆) |
| SERIES               |                              |
| WHOLE CELL VACCINE  | 13 patients (1 alive and well; length of follow-up indicated by ●) |

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Figure. Comparison of lysed and whole cell vaccine series with control series in terms of the disease-free interval from amputation to pulmonary metastasis.
patients appear longer. In addition, all patients in the control group have been followed well over two years, whereas only six of the nine disease-free patients in the lysed cell series have had long-term follow-up. Therefore, this paper is intended as a preliminary report only. There is, however, a statistically significant difference between the response rates of the two vaccine groups which suggests clinical evidence of immunologic manipulation of a human tumor.

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Challenge to Sacred Cows

The implication that general acceptance of a therapeutic procedure by physicians in a given era constitutes irrefutable proof of effectiveness is not tenable. If this were true, we would still be bound to the mummy dust, unicorn’s horn, leeching, purgatives, blood letting, and mustard plasters universally endorsed by our forebears. We must constantly offer challenge to all our sacred cows, so that our patients may be afforded the highest-quality care at the most reasonable cost. —Charles G. Moertel, M.D., and David L. Ahmann, M.D. Correspondence. New England Journal of Medicine 286: 1158, 1972.