



**OSTEOSARCOMA
ALLIANCE**



Guidelines: Proposal must benefit the osteosarcoma patient and be a new project or distinct portion of an ongoing larger project for which results can be expected in 12 months. At the completion of 12 months, results must be made available to share - regardless of succeed or fail outcome. The recipient must be available to present work underway and completed at the FACTOR conference in 2019. Fund may not be used for the formation of new organization or used for planning stages of research or other initiative. Presentation of check and tour of facility by MIB Agents is requested.

Please fill out the form below, proposals will be submitted as a layman's summary and are limited to front and back of this page. Completed RFP will be available for the public to view on MIB Website and social media so the public can vote. Deadline for submission is April 20, 2018. Email to info@MIBagents.org

Name: Matteo Trucco, MD

Organization: University of Miami-Sylvester Comprehensive Cancer Center

Email: Redacted

Phone: Redacted

Initiative Name: Clinical trial of disulfiram to overcome chemotherapy resistance in sarcomas.

Amount Requested: \$100,000 for purchase and compounding of disulfiram, and for ALDH testing.

Desired Impact: To prevent relapses by overcoming resistance to chemotherapy.

Projected Milestones: By early 2019, we anticipate having the trial approved and ready to enroll patients.

I agree to Guidelines: Matteo Trucco

Background: Adding chemotherapy to surgery greatly improved the survival of children and young adults with osteosarcoma. The treatments used, however, have significant short and long-term side effects, and the benefits are mostly for patients with localized disease. Even if the osteosarcoma seems to respond to therapy and whatever tumor seen is removed surgically, the tumors often come back. One explanation for this that there may be a small population of sarcoma cells that are resistant to chemotherapy but then grow recurrent (relapsed) tumors (Figure 1). These cells are often referred to as Cancer Stem Cells (CSCs). A way of identifying CSCs in several sarcomas including osteosarcoma is isolating the cells that have high levels of the enzyme Aldehyde Dehydrogenase (ALDH).¹⁻³ Recent data suggests ALDH is responsible for the resistance to chemotherapy seen in CSCs.⁴⁻⁷ ALDH's job in cells is to break down aldehydes. Key chemotherapeutic drugs such as cyclophosphamide and ifosfamide are aldehydes. ALDH has also been associated with resistance to the many other chemotherapy drugs used to treat sarcomas, including cisplatin, doxorubicin, gemcitabine and docetaxel. This is thought to be because of ALDH's ability to inhibit reactive oxygen species (ROS) production, which is essential to how some chemotherapy drugs and radiation kill cancer cells (Figure 2).^{5,8}

We recently completed a clinical trial of the mTOR inhibitor temsirolimus combined with liposomal doxorubicin for relapsed sarcomas showing that the combination prolonged survival more than either drug alone and that ALDH levels went down in the tumors of the patients that benefitted from the treatment (*manuscript submitted*). Blocking mTOR in cells has been shown to decrease ALDH levels, and subsequently increases osteosarcoma cells' sensitivity to chemotherapy-induced ROS and decreases their ability to form metastasis.⁸ While mTOR inhibition appears to have some effect on ALDH-expressing sarcomas, ALDH can be blocked more directly and effectively with a medication called disulfiram. Disulfiram, originally developed as an anti-alcoholism drug over 60 years ago, specifically blocks ALDH, which is also necessary to breakdown alcohol. Several studies have shown disulfiram's ability to inhibit ALDH in cancer cells such as osteosarcomas, reversing their resistance to chemotherapy and killing them.^{5,8-10} Disulfiram is safe and patients can take it for years.^{11,12} Clinical trials testing disulfiram combined with chemotherapy are currently underway for breast, prostate, lung, and pancreatic cancer, among others. We propose a phase I clinical trial of disulfiram combined with gemcitabine/docetaxel for

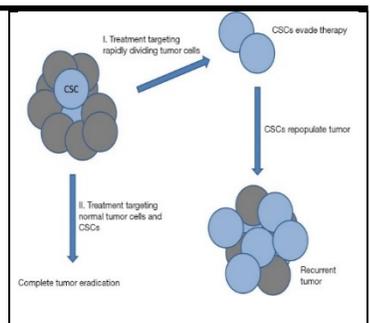


Figure 1. Depiction of CSC giving rise to recurrent tumor (Clark and Palle Annals of Translational Medicine 2016)

the treatment of children and young adults with relapsed osteosarcoma, Ewing sarcoma, rhabdomyosarcoma and other sarcomas. We hypothesize that adding disulfiram to chemotherapy will make sarcoma CSCs sensitive to the chemotherapy leading to better and sustained responses to treatment.

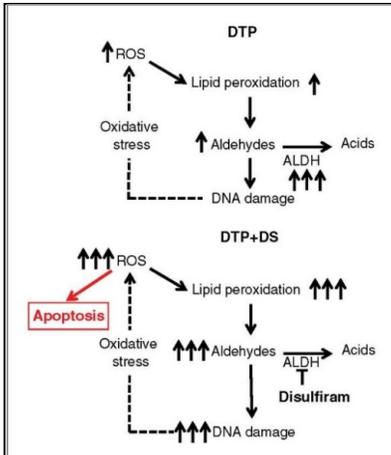


Figure 2: Proposed role of ALDH in resistance (top) and effect of disulfiram (bottom). DTP = drug-tolerant population; DS = disulfiram; ROS = reactive oxygen species; (Raha et al. Cancer Research 2014)

Rationale: Laboratory studies have shown disulfiram is toxic to sarcoma cells and make them sensitive to chemotherapy. Gemcitabine/docetaxel is a well-established and well-tolerated regimen for the treatment of several different relapsed sarcomas.^{13,14} There is evidence that disulfiram makes cancer cells more sensitive to both gemcitabine and docetaxel, supporting a clinical trial of the proposed combination.^{9,10} Disulfiram, however, has yet to be tested in patients with sarcomas. At the Sylvester Comprehensive Cancer Center (SCCC), we have a very active adult and pediatric sarcoma program, including dedicated oncologists, surgeons, pathologists and interventional radiologists, as well as very active adult and pediatric clinical trial programs. We have completed multiple clinical trials focusing on sarcomas. We have significant experience in clinical trials that include minimally invasive serial tumor biopsies to test response to our treatments. Furthermore, we have the reagents, experience, and resources to measure precisely ALDH levels in patient tumor samples.

Research Strategy:

Specific Aim1: Determine the safety and tolerability of disulfiram in combination with gemcitabine/docetaxel in pediatric and young adult patients with relapsed or resistant sarcomas. We will conduct a clinical (phase 1, 3+3, dose escalation) trial of disulfiram in combination with standard dose gemcitabine/docetaxel. For safety, the initial dose of disulfiram in this combination will be 80% of the standard dose (240mg/m²).

If the initial dose level is determined to be safe, we will then give 100% standard dosing (300mg/m²). If 80% dosing is too toxic, a 50% dose (150mg/m²) will be tried. The standard gemcitabine/docetaxel regimen to be used is gemcitabine (900 mg/m²) on Days 1 and 8 and docetaxel (75 mg/m²) on Day 8 of a 21-day cycle. Disulfiram will be administered once daily by mouth on Days 1 to 21. Inclusion criteria includes age 1-40 years old with a diagnosis of relapsed or refractory sarcoma who have adequate organ function. Adult patients (> 18 years old) must agree to minimally invasive tumor biopsy prior to treatment and after cycle 2, if deemed safe. Tumor biopsies will be optional for patients <18 years old. Patients must abstain from alcohol consumption and cannot take medication known to interact with disulfiram. An appropriate “washout” period from previous therapy must elapse prior to beginning treatment on this trial. Side effects, physical exam, and laboratory tests will be monitored to determine what dose of disulfiram is safe to give with gemcitabine/docetaxel using the National Cancer Institute - Common Terminology Criteria for Adverse Events (NCI-CTCAE), Version 4.0. Patients can continue treatment on the study for a maximum of 12 months as long as the patients appear to be benefiting from the treatment and they wish to continue on the study. Response to therapy will be assessed every 6 weeks with radiographic imaging and measured using Response Evaluation Criteria in Solid Tumors (RECIST) criteria.

Specific Aim 2: Test how effective Disulfiram is in eliminating Aldehyde Dehydrogenase (ALDH) expressing cancer stem cells and treating relapsed/refractory sarcomas. Minimally invasive core-needle biopsies of tumors will be performed by interventional radiology at two time points: 1) prior to beginning therapy and 2) after 2 cycles of treatment. Tumor tissue collected on patients enrolled on the study will be measured for ALDH levels. Tissue samples will be stored in liquid nitrogen until testing. On each patient, the two samples will be analyzed at the same time for more accurate comparison. Samples will be broken up into single cells and the ALDEFLUOR[®] reagent (Stem Cell Technologies, Vancouver, BC) will be used to label the ALDH. The cells will then be analyzed using a machine called a flow cytometer to measure the amount of ALDH in each cell and identify the CSCs population. We routinely performs this assay on healthy and cancer cells and have standardized protocols and controls for these tests. The technicians performing this testing will not be aware of whether the patients are responding to treatment to avoid bias. The ALDH measurements in pre- and post-treatment tumor samples will be sent to the team running the clinical trial who will see if ALDH levels match up with response to therapy. This will be helpful to determine what patients are likely to benefit from this therapy moving forward.

Innovation/Significance: While not exclusive to osteosarcoma, the proposed study is the first step in developing disulfiram as an addition to the treatment of sarcomas. This clinical trial proposes a novel strategy for targeting a source of resistance to chemotherapy thought to allow relapsed disease. It represents a major step toward more targeted, less toxic and more effective treatments for children and young adults suffering from sarcomas. By targeting CSCs with a well-studied drug, we can potentially eliminate the key cells responsible for relapses and metastases, safely. The proposed studies measuring ALDH levels pre- and post-therapy are essential for confirming that disulfiram is really inhibiting ALDH and that ALDH levels predict which patients are likely to benefit from this type of therapy. The findings from this study will be used to secure additional funding from the NIH and other foundations to support expanded trials in specific sarcomas including osteosarcoma.

References:

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