# Assessing the effects of c60oo on human cancer proliferation *in vivo*

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## Background

The extreme life extension in Wistar rats given c60oo reported by Baati et al. is well known in the LE community. There is precedent for LE from other fullerene compounds[1,2] and at least three placebo controlled lifespan experiments evaluating c60oo in rodents are currently underway. A less well known aspect of Baati's experiment is the effect on age-related cancer incidence. Baati's control rats all showed evidence of tumors at necropsy, as is typical for rodents, but despite their advanced age, none of the treatment group had any tumors[3]. This raises a number of questions as to mechanism of action: Is the effect due to prevention of the transformation of a normal cell to a viable cancer cell? Is it due to a reduction in proliferation and metastasis? Is it a combination of these effects? Is it preventing one while promoting the other? What would be the effect on human cancers?

The last two questions are of particular relevance to the use of c60oo by humans. There are reasons to think that c60oo could be pro-cancer in humans, based on its known property as a potent antioxidant agent and its apparent enhancement of oxygen utilization *in vivo*:

- In clinical trials, various antioxidants (beta-carotene, vitamin E, selenium) have led to higher cancer incidence, rather than lower[4].
- Cancer cells are oxidatively challenged, and upregulate endogenous antioxidant defenses to compensate. An external antioxidant might enhance survival of these cells. In a 2013 paper, James Watson suggested that antioxidant compounds may have caused more cancers than they have prevented[5].
- The tumor environment is hypoxic, thus a more efficient utilization of oxygen could lead to improved tumor survival[6].

We propose a series of xenograft experiments in which a well-characterized human cancer cell line will be implanted in mice with and without c60oo treatment. The mice used in these experiments have a genetic defect in their immune system that prevents them from rejecting human cells. This model is commonly employed in cancer research as a superior alternative to *in vitro* testing, and will address an important gap in our knowledge regarding the effect of c60oo on cancer proliferation.

## Research plan

The objective of the present study is to assess the effect of c60oo administration on human leukemia proliferation *in vivo* using a modified version of the protocol described by Lehne et al[7]. Briefly, the severe combined immunodifficient (SCID) mouse is a routinely used model organism with impaired T and B cell production, which makes it a suitable host for foreign tissue transplants, including cancer. We will dose SCID mice with the human acute myelogenous leukemia line KG-1a. We will perform frequent blood analysis using a highly

sensitive flow cytometry assay to track relative and absolute proliferation of KG-1a *in vivo*. The study will feature two control groups, one treated with phosphate buffered saline, and the other treated with olive oil. Low (1 mg/kg), medium (4 mg/kg), and high (8 mg/kg) treatment doses of c60oo will be administered to three experimental groups. For all conditions, treatment will occur every two days for six days, then once per week thereafter. Routine chemistries will also be performed periodically to assess pancreatic, liver, and renal function. See “Table 1” for a summary of the different cohorts.

| | Qty | Description |
|---|---|---|
| PBS control | 5 | Control group dosed with KG-1a and treated with sterile saline. |
| Olive oil control | 5 | Control group dosed with KG-1a and treated with olive oil. |
| Low dose c60oo | 5 | Experimental group dosed with KG-1a and treated with 1 mg/kg c60oo. |
| Medium dose c60oo | 5 | Experimental group dosed with KG-1a and treated with 4 mg/kg c60oo. |
| High dose c60oo | 5 | Experimental group dosed with KG-1a and treated with 8 mg/kg c60oo. |

**Table 1: Study treatment conditions.** Two control groups will be treated with PBS or olive oil. Low, medium, and high dose c60oo groups will be treated with 1 mg/kg, 4 mg/kg, or 8 mg/kg, respectively. For all conditions, dosing will occur every two days for six days, then once per week thereafter.

## Specific aims

1. To determine the effects of c60oo on leukemia proliferation. The main goal of this study is to determine whether c60oo increases, decreases, or has no effect on cancer proliferation. We have optimized highly sensitive and specific analytical methods to accurately track human cell populations within a mouse host. We will use these methods to determine relative and absolute leukemia proliferation in the context of c60oo treatment.

## Methods

*Cell culture*

The human acute myelogenous leukemia line KG-1a is cultured as previously described[7]. Briefly, cells are cultured in Iscove's Modified Dulbecco's Medium supplemented with 20% fetal bovine serum. Culture medium is renewed twice per week. Prior to *in vivo* study, KG-1a will be scaled and 10^7 exponentially growing KG-1a cells will be injected by teil vein into each study animal.

*C60-olive oil solution preparation*

C60oo is prepared as previously described[8]. Briefly, 50mg c60 (purity >99.8%) from SES Research Corporation is dissolved in 10 mL virgin olive oil by stirring for 2 weeks at room temperature in the dark, then centrifuged at 5,000 G for 1 hour. The resulting supernatant is passed through a 0.25um porisity filter. Experimental animals will receive C60oo by intraperitoneal injection. Controls will receive either phosphate buffered saline or olive oil by intraperitoneal injection.

*Flow cytometry*
10uL peripheral blood is incubated with 1uL of antibody for 20 minutes at room temperature in the dark. 89uL of lysis buffer is added and the sample is incubated at room temperature in the dark for 10-20 minutes before being run on the Accuri C6 flow cytometer.

*Husbandry*
This study consists of a total of 25 SCID mice on a 12 hour light/dark schedule within a positive pressure clean room. Within the clean room, all study animals are housed in individually ventilated cages with 5-10 mice per cage. The mice are fed sterile pelleted mouse diet and given sterile water ad libitum.

*Toxicity*
Chemistry analysis and complete blood counts will be obtained monthly according to manufacturer recommendations.

**Relevance of this work to the life extension movement**
C60oo has the hallmarks of being the most potent life extending agent yet discovered, and is already in use by a sizable and growing cohort. However, there is reasonable skepticism and an unwillingness by others to use a compound for which long term safety in humans is unknown. Further, many life extensionists who themselves use c60oo are hesitant to recommend it to others for the same reason. The largest concern is that it might promote the growth of an existing indolent tumor. The proposed research would shed light on this important issue, and would allow wider use of the compound, or alternatively, a more accurate risk assessment, depending on the outcome.

C60oo can be thought of as a bridge to more sophisticated regenerative medicine. The bridge would take two forms: First, this compound fills several unmet medical needs, and would be immediately life preserving in certain medical conditions such as many forms of heart failure, severe asthma, or COPD. For others it will be life-promoting, essentially acting as a curve-squaring compound at the very least. We suspect that it will be brain and functionality-preserving in ischemic stroke[9], without negatively effecting hemorrhagic stroke. There is precedent for other fullerene analogs being neuroprotective[10]. The second way in which C60oo could act as a bridge to damage-repairing technologies involves the modification of widespread beliefs regarding the inevitability of aging and death. There are many wealthy philanthropists capable of funding SENS at levels viewed as sufficient, but they do not choose to do so. While it may be possible that they are simply unaware of it, a more likely reason is that they do not believe it is possible. A compound in widespread use that was known to be life extending in mammals would lead many, particularly among the philanthropic community, to question their beliefs, and may encourage some of them to loosen their purse-strings.

**Facility**
Ichor Therapeutics, Inc. is a privately held pre-clinical biotechnology company with corporate offices, R&D facilities, and a vivarium in Syracuse, NY. Capital assets relevant to the present proposal include an Accuri C6 flow cytometer (used to quantify relative and absolute leukemia levels within peripheral blood), Heska HemaTrue automated hematology analyzer (used to determine completed blood counts), SCID vivarium (double positive pressure clean room for housing immunocompromised mice), and a mammalian cell culture laboratory (used to grow and maintain leukemia cell lines).

## Budget

This budget will support 6 months of focused research operations to assess the effects of c60oo administration on leukemia proliferation *in vivo*.

| | July | August | September | October | November | December | SUBTOTAL |
|---|---|---|---|---|---|---|---|
| ***Research variable costs*** | ***$ 6,064*** | ***$ 389*** | ***$ 389*** | ***$ 389*** | ***$ 389*** | ***$ 389*** | $ 8,009 |
| Cons. & reag. (plastic, media, c60oo, etc) | $ 2,250 | $ - | $ - | $ - | $ - | $ - | |
| Analytics (antibodies and panels) | $ 3,200 | $ 250 | $ 250 | $ 250 | $ 250 | $ 250 | |
| Cell line (KG-1a) | $ 475 | $ - | $ - | $ - | $ - | $ - | |
| Husbandry (mice, food, bedding, staff, etc) | $ 139 | $ 139 | $ 139 | $ 139 | $ 139 | $ 139 | |
| ***Capital equipment*** | ***$ 3,250*** | ***$ -*** | ***$ -*** | ***$ -*** | ***$ -*** | ***$ -*** | $ 3,250 |
| Chemistry analyzer (Idexx VetTest 8008 series) | $ 3,250 | $ - | $ - | $ - | $ - | $ - | |
| ***Salaries and facilities*** | ***$ 980*** | ***$ 980*** | ***$ 980*** | ***$ 980*** | ***$ 980*** | ***$ 980*** | $ 5,880 |
| Labor (part-time cell biologist and husbandry specialist) | $ 750 | $ 750 | $ 750 | $ 750 | $ 750 | $ 750 | |
| Rent and utilities | $ 80 | $ 80 | $ 80 | $ 80 | $ 80 | $ 80 | |
| Misc | $ 150 | $ 150 | $ 150 | $ 150 | $ 150 | $ 150 | |
| ***SUBTOTAL*** | $ 10,294 | $ 1,369 | $ 1,369 | $ 1,369 | $ 1,369 | $ 1,369 | ***$ 17,139*** |
| Running total | $ 10,294 | $ 11,663 | $ 13,032 | $ 14,401 | $ 15,770 | $ 17,139 | |
| Budget by quarter | | | $ 13,032 | | | $ 4,107 | |
| | Q1 | | | Q2 | | | |

**Table 2: Detailed budget.** Research equipment, laboratory space, and vivarium housing will be provided by Ichor Therapeutics, Inc. at cost. Labor expense is also being significantly subsidized by the company, so labor expenditures for two part-time researchers is reduced to only $750 per month.

## References


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