Meeting minutes: SCLC Consortium WebEx

October 4, 2018 @ 1:00pm ET

Announcements/Updates

• Three more grants funded through the U01!
  o Mark Krasnow-Stanford
  o Kwon Park-UVA
  o Alissa Weaver and Christine Lovly-Vanderbilt

• Detection and Prevention PAR is no longer active

Title: Enhancing Small Cell Lung Cancer Therapy with Pharmacological Ascorbate

Dr. Bryan Allen- Dept of Radiation Oncology, University of Iowa

• Small Cell Lung Cancer
  o Epidemiology
    ▪ 29,654 estimated cases in the US in 2018
    ▪ 14% of lung cancer
    ▪ Incidence is decreasing with smoking prevention
      o 95% of cases are attributable to cigarette smoking
      o Incidence in women is increasing with a male to female incidence ration of around 1:1
  o Median age at presentation: 64 years old
  o Screening with LDCT
    ▪ Currently deemed not useful per NCCN guidelines
      • Aggressive: Develop of symptomatic disease in between annual scans
  o Presentation
    ▪ Imaging-large hilar mass with bulky mediastinal lymph nodes
    ▪ Symptoms: cough and shortness of breath
    ▪ Associated paraneoplastic syndrome
      • Lambert Eaton Syndrome: 1-2%
        ▪ Proximal leg weakness due to antibodies against voltage gated calcium channels
    ▪ Ectopic Hormone Reduction
      • Antidiuretic hormone (vasopressin)-10%
        ▪ Hyponatremia: fatigue, headaches, seizures
      • Adrenocorticotropic hormone (ACTH)-2-5%
        ▪ Hirsutism, hypertension, hyperpigmentation, glucose intolerance, abdominal obesity
  o Staging
    ▪ Historical VA Lung Study Group
      • Limited stage (1/3): disease confined to the ipsilateral hemithorax
        ▪ May include contralateral mediastinum and ipsilateral supraclavicular disease
      • Extensive stage disease (2/3): beyond the ipsilateral hemithorax to other lung, lymph nodes on other side of chest, or distant organs
    ▪ AJCC TNM Staging System
      • Limited stage correlates to stage I-III (any T, any N, M0) that can be safely treated with definitive radiation and chemotherapy
      • Extensive stage correlates to stage IV disease and T3-T4 disease due to multiple lung nodules that are too large or extensive to be treated with definitive radiation and chemotherapy
• **Traditional Treatments and Outcomes**
  
  **Limited stage**
  
  - Chemo-RT → prophylactic cranial irradiation (PCI)
    - Cisplatin + Etopiside + Radiation
      - Chemo-responsive: response rates of 80-90%
        - Rarely sustained (median 6-8 months)
      - Radiation dosing
        - QD: 60-70 Gy at 1.8-2 Gy/fx
        - BID: 45 Gy at 1.5 Gy/fx
  
  - Timing
    - Begin RT as soon as possible (cycles 1-2)
      - 50-60% relative improvement in 3 years overall survival compared to starting RT after cycle 2 (oncologist 2004;9;665)

  - Surgery option for early stage

  - Outcomes
    - Median OS: about 24 months
    - 5 year OS: 26%
    - Thoracic failure rate: 35%

  **Extensive Stage**

  - Typically managed with chemo (Cisplatin+etop)
  - Palliative radiation for symptom management and consolidation (?)
  - Outcomes
    - Median OS: 5-9 months
    - 5 yr OS <5%

• **Hypothesis**

  - We can manipulate the metabolic oxidative stress differences between cancer cells and normal cells to improve small cell lung cancer therapeutic response

• **Oxidative Stress**

  - Disturbance in the prooxidant-antioxidant balance in favor of the former that potentially leads to an accumulation of oxidative damage to critical biomolecules

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**Antioxidants**

- Glutathione
- Vitamin E (α-Tocopherol)
- Coenzyme Q (Ubiquinol)
- Uric Acid
- Lipid Acid
- Vitamin C (ascorbate)
- Enzymes (SOD)
- Deferoxamine

**Prooxidants**

- Create reactive oxygen species
- Hydroxyl radical
- Superoxide
- Hydrogen peroxide
- Organic peroxides
- Peroxynitrites
- Lipid peroxidation products

**Oxidative Damage**

- Tissue Injury, Inflammation, Cell Death
• Reactive Oxygen Species are Formed Predominantly in Mitochondria

• Cancer Cells have Increased Oxidative Stress Relative to “Normal Cells”

• Radiation and Chemotherapy also Induce Oxidative Stress

• History of Vitamin C as a Cancer Therapy
  o 1970's: Linus Pauling showed that high dose ascorbate (10g IV then 10g PO) increased survival 4x in a variety of cancers
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- 1985: Two randomized trials demonstrated no difference in survival between patients receiving placebo or 1g oral ascorbate
- 1985: Two randomized trials demonstrated no difference in survival between patients receiving placebo or 10g oral ascorbate
- 1990’s: Hugh Riordan demonstrated *in vitro* that many tumor cell types die when exposed to high concentrations of ascorbate (400mg/Dl) while normal cells are unaffected
- 2005 Mark Levine (NIH) demonstrated that effective concentrations of ascorbate could not be achieved by oral administration alone

- Oral vs. IV Ascorbate

**Oral ascorbate levels in blood**

**I.V. ascorbate levels in blood**

- Pharmacological Ascorbate Increases NSCLC Sensitivity to Radiation and Chemotherapy *in vitro*

- Pharmacological Ascorbate Enhances NSCLC Radiation and Chemotherapy Sensitivity *in vivo*

- Ascorbate Levels are Increased in Plasma and Tumor
  - Humans and hamsters are the only mammals that do not make their own Vitamin C, so mice already have a background concentration of Vitamin C in their plasma
- H2O2 Mediates Ascorbate Cancer Cell Toxicity

- Redox Active Metal Ions Mediate Ascorbate Cancer Cell Toxicity

- Iron Metabolism and the Redox Active Labile Iron Pool
  - Lung tumors have increased labile iron pools relative to adjacent normal lung tissue
• Iron Regulation Influences the Differential Susceptibility of Cancer vs. Normal Cells to Pharmacological Ascorbate

Cellular Expression

- Transferrin Receptor (TfR)
- Actin
- Ferritin (Ft-H)
- Actin
- Ferritin-Large (Ft-L)
- Actin

Patient Samples

- TfR
- Actin

Clonogenic Rescue

- H1299

• Available Labile Iron Pools Influence Cell Sensitivity to Pharmacological Ascorbate
  - Knock down transferrin receptor to make cells that are sensitive to Vitamin C, resistant to Vitamin C
• Pharmacological Ascorbate Cancer Cell Selectivity Model

![Diagram of mitochondrial oxidative stress and redox signaling](image)

• Phase 2 Clinical Trial of Pharmacological Ascorbate in Stage IV NSCLC
  - Give carbo+etop every three weeks (standard therapy) AND ascorbate IV twice a week

• Interim Results of Pharmacological Ascorbate Lung Trial

![Graph showing tumor burden over time](image)

• Redox Active Copper
  - Transition metal ions like copper (Cu) and Iron (Fe) can participate in oxidation reactions
    - $\text{M}^{(-n)} + \text{H}_2\text{O}_2 \rightarrow \text{M}^{(+n)} + \text{OH}^- + \text{HO}$
  - Copper ATSM (copper(II)bis(thiosemcarbazone): previously used in clinical trials to treat ALS and Parkinson’s disease
    - CuATSM preferentially concentrates in hypoxic tissues releasing its copper atom after entering cells

• Overview of CuATSM Uptake
  - Hypoxic conditions: Cu(II)ATSM is reduced to Cu(I)ATSM is reduced to Cu(I)ATSM
    - The complex becomes unstable and copper is released and may participate in oxidation reactions
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- CuATSM Enhances SCLC Radiation Sensitivity in Hypoxic Conditions

- Pharmacological Ascorbate Enhances SCLC Sensitivity to Radiation

- Pharmacological Ascorbate Enhances Survival in SCLC Xenografts Treated with $^{90}$Y-DOTATOC

- Future Directions
  - SCLC xenograft studies assessing combinations of CuATSM, radiation therapy, chemotherapy, and pharmacological ascorbate
  - Clinical trial in SCLC combining pharmacological ascorbate with cisplatin and etoposide in extensive stage and pharmacological ascorbate combined with radiation, cisplatin and etoposide in limited stage
Questions:
- What was the rationale for choosing DOTATOC for combination therapy for SCLC?
  - May not be the most active agent but it was available from a collaboration with the nuclear medicine department.
- NCI did large number of combined radiotherapy/chemotherapy studies. Helped with local disease but the problem was with metastatic disease. What is the appropriate preclinical model to look at metastatic disease? Does it look like there is any other evidence that this could deal with metastatic disease?
  - For metastatic disease, combine chemo and ascorbate. For overcoming resistance to chemo, would have to do initial animal studies combining chemo with Vitamin C and combining chemo with radiation.
- Why in some cases does the ascorbate seem ok and then in other cases not. What are the underlying factors of why it is not consistent?
  - Some cell lines are not sensitive. Believe it is related to amount of redox active metal inside the cell.
- Thoughts on effects of radiation generating immune response and how can it be evaluated in a preclinical fashion to see if whether combining this would lead to a SCLC immune response?
  - World Lung result was without radiation but there are multiple models that show there is synergy between radiation and immune response.

Reminders:
- Next Call: November 1st from 1:30-2:30pm
  - Dr. Christopher Vakoc-Cold Spring Harbor Laboratory
- Annual Spring Meeting will be evening of April 3rd–April 5th 2019
  - Planning committee: Charles Rudin, JT Poirier, Trudy Oliver, Julie George and Taofeek Owonikoko