How to measure the effectiveness of therapy by measuring oxygenation of the target tissues

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Disclosures

• Scientific Director of Clin-EPR, LLC (wife President) which is the commercial source for dissemination of technology originating in the EPR Center at Geisel

• No direct involvement of the company in the research to be presented

• Have some IP related to EPR oximetry

• Not a real company, in the sense that any profits are used to support the EPR Center

• One focus of my career is on measurements of oxygen in vivo and therefore my input is influenced by this focus
What should we measure to determine the effectiveness of RBC based therapy?

• Goal is to increase the amount of oxygen at the site of interest in the expectation that this will affect outcomes

• Perhaps the best way to do this is to measure the oxygen content at the site

• Need to be able to make the measurements in the type of population for which the therapy is intended

• Would like to be able to make these measurements both for initial evaluation of the preparation and also for the effectiveness in individual patients

• Also highly desirable get the information both dynamically and repeatedly

• It turns out that most of the techniques available to measure oxygen in tissues fall short of meeting these needs
Some modulations of presentation based on prior presentations

• While this presentation is organized about clinical applications, the same concepts apply to preclinical studies as well
• An important theme of the previous presentations is the need for data, because our hypotheses and reasoning are imperfect
• These considerations reinforce the importance of actually measuring the oxygen in the tissue
• This does not diminish the value of other measures of functionality and pathophysiology
• But we also should remember that the ultimate determinant of efficacy is outcomes.
Existing methods to measure oxygen in vivo

• Should differentiate among:
  – Methods that directly measure O₂ in tissues
  – Methods that measure O₂ in the vascular system
  – Methods that measure parameters that reflect O₂ via plausible mechanisms

• Need to consider applicability in the real world of clinical medicine: are they available today and can the measurements be made under usual clinical conditions

• Also useful if they can be used similarly in animal models so that the results can be better related to clinical uses
Methods that measure parameters that reflect O₂ via plausible mechanisms

- Hypoxic markers - nitroimidazole derivatives & [Cu]-ATSM
- Indicators of redox state
- PET based on metabolism of glucose analogs
- BOLD MRI
- MRI perfusion and diffusion
- MRI spectroscopy of hypoxia related molecules

Note that these are the techniques that are most likely to be available clinically and preclinically

BUT, they do not measure oxygen directly so need to understand what they do measure, and whether it is the information needed for decision-making
Methods that measure $O_2$ in the vascular system

- NIRS
- Blood gases

- These are also available clinically
- They also do NOT necessarily relate directly to the oxygen in the tissues of interest
Methods that directly measure $O_2$ in tissues

- Oxygen electrode
- OxyLite and related optical methods based on oxygen quenching of light emission
- Fluorine hydrocarbon NMR
- EPR oximetry
Methods that directly measure $O_2$ in tissues

- **Oxygen electrode**
- Considered “gold standard”
- Modest limitations due to possible injury at time of measurements
- Not practical to make repeated measurements and continuous measurements are challenging.
- **No longer commercially available**
Methods that directly measure O$_2$ in tissues

- **OxyLite and related optical methods based on oxygen quenching of light emission**
- Can provide measurements in track similar to oxygen electrode
- Similar problem of local perturbations
- **Not clinically available**
Methods that directly measure O₂ in tissues

- Fluorine hydrocarbon NMR
- Requires direct injection into site
- Can make repeated measurements
- Has not had clinical translation
- Some promising other NMR techniques are under development
Methods that directly measure $O_2$ in tissues

- EPR oximetry
- Requires one-time injection of oxygen sensitive material into the tissue of interest
- Can be used to make continuous measurements and repeated measurements over time
- Widely used successfully in preclinical models
- Clinical measurements underway using India Ink and PDMS coated other materials
Oximetry: Peripheral Vascular Disease
Long term measurements of the same site in the human foot

- **Baseline**
- **Compression**
- **Recovery**

**Variables:**
- $\text{pO}_2$ [mmHg]
- **Years**

Graph shows data points over time, indicating measurements of $\text{pO}_2$ at different years, with markers for baseline, compression, and recovery conditions.
In Vivo use of EPR Oximetry

Extensive successful use in preclinical studies in animals ranging from mice to pigs and essentially all tissues. Measurements human subjects with EPR (dosimetry and oximetry) have been made at several institutions in the USA (Dartmouth, Emory, Yale, Rochester, Dana Farber) and in Korea, Japan, & Belgium.

Ability to make measurements both continuously and repeatedly demonstrated.

May be especially useful to determine the circumstances under which when more widely available but less intrinsically specific techniques such as MRI or PET will provide useful information on tissue oxygen.
SUMMARY

• Adequate evaluation of the utility of red cell preparations should include direct demonstration of their effectiveness in improving oxygenation in the target tissues, including subjects that are representative of the population for which the therapy will be applied.

• This is a technical challenge to do with high confidence.

• Techniques are available to do this and should be utilized in both preclinical and clinical settings.

• It would be especially valuable to be able to monitor oxygen in the individual subjects receiving the therapy and to be able to do this under clinically applicable conditions.

• We would be very glad to collaborate with others to make oxygen measurements in their experimental systems.
THANK YOU
ANY QUESTIONS?