Longitudinal, Non-invasive and Quantitative Measurement of Vascular Physiology *in vivo* from Irradiated Head And Neck Cancers using Diffuse Optical Spectroscopy

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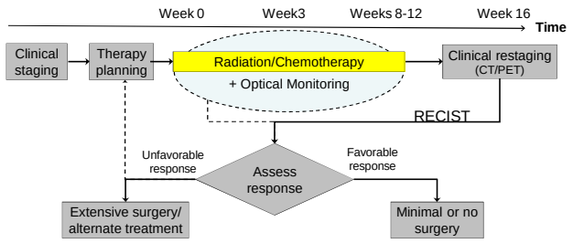
ABSTRACT

Longitudinal measurements of diffuse reflectance were obtained *in vivo* from head and neck cancers (HNC) using fiber-based spectroscopy in mouse and human tumors that were undergoing curative radiotherapy. These spectra were quantified using an inverse Monte Carlo model to extract optical endpoints of vascular oxygenation (SO_2) and hemoglobin content (THb). Analysis of the extracted optical endpoints indicated that measured variance across subjects was higher than within subjects and that exposure to radiation caused increases in irradiated tissues, relative to non-irradiated controls. In both mice and humans, subjects with long-term disease free survival (of the primary treated site) showed sustained increases of SO_2, per subject, relative to baseline levels, across time.

INTRODUCTION AND BACKGROUND

- The growth rate, metastatic potential and response to radiation therapy of HNC has shown to be dependent on changes in tumor oxygenation levels [1]
- Clinical tools to sense tissue oxygenation include both invasive (such as the pO_2 electrode and immunohistochemical analysis of biopsied tissue) and non-invasive methods such as MR or radio-labeled imaging and spectroscopy
- The clinically invasive methods cannot be used routinely, while the non-invasive techniques are specialized, expensive, or time-consuming for daily use
- Optical methods could provide real-time sensing of tumor hypoxia, metabolism and angiogenesis in clinical settings

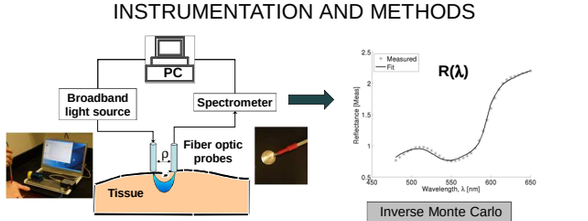
CLINICAL MANAGEMENT OF HNC



MOTIVATION AND HYPOTHESIS

- Pre- and post-treatment measurements of tumor hypoxia using pO_2 electrodes and PET have shown that improved oxygenation related to better outcomes [2,3]
- In order to personalize and individualize cancer treatment, it will be necessary to evaluate tumor physiology repeatedly during treatment
- Diffuse correlation and reflectance spectroscopy has been used to monitor changes in blood flow and oxygenation at neck nodes of HNC patients but did not highlight differences between responders and partial responders [4]
- Here, we use optical spectroscopy at the primary site to investigate changes in oxygenation during radiotherapy in both animal and human studies

INSTRUMENTATION AND METHODS



The concentrations of oxy- (HbO_2) and deoxy-hemoglobin (dHb) are obtained from the absorption spectrum to compute

THb = HbO_2 + dHb

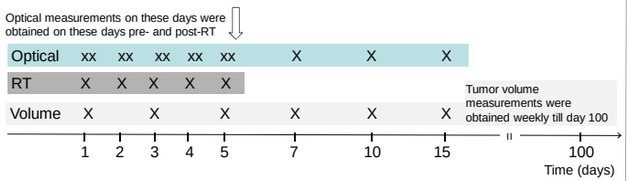
SO_2 = 100*HbO_2/THb

$$< \mu_{s'} >= \frac{\sum_{k=1}^{N} \mu'_s(\lambda_k)}{N}$$

ANIMAL EXPERIMENTS

Study design:

- 42 Nude mice inoculated with FaDu cells in flank
- Treatment started when tumor volume ~ 100-400 mm^3
- Animals anesthetized by isoflurane + O_2 breathing
- Optical probe randomly placed on 3-5 sites/animal



RESULTS

Table 1 shows the breakdown of the animal groups based on a 100 day follow-up on the treated animals. Animals that were tumor free at the end of the 100 day period were classified as complete responders (CRs). Animals that showed local recurrences were classified partial responders (PRs). **Fig. 1** shows the survival curves for the animal groups in Table 1.

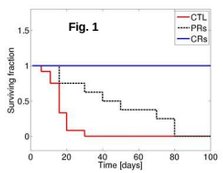
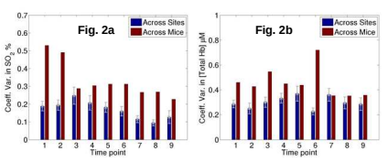


TABLE 1: Treatment response categories

Group	Dose		TOTAL
	5x7.5 Gy	5x 9.5 Gy	
Untreated (controls)	5	7	**12**
Treated (local response)	4	14	**18**
Treated (local recurrence)	6	2	**8**
Censored	2	2	**4**
TOTAL	**17**	**25**	**42**

As optical measurements were obtained from multiple sites across each animal, we could compute the coefficient of variance (CV, defined by the ratio of the standard deviation to the mean) for optical measurements across sites and across animals, for each time point measured. **Fig 2a** and **2b** show the CV in the measured optical saturation and total hemoglobin concentration, respectively. The error bars on the computed CV across sites represents the standard error in the CV values across animals.



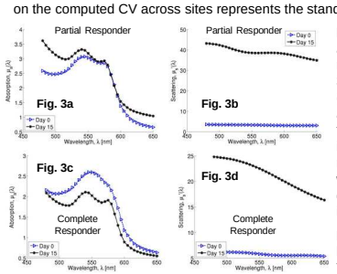


Fig. 3a-3d show the extracted optical absorption and scattering spectra for one representative animal from the PR (3a and 3b),and CR (Fig. 3c and 3d) group at baseline (Day 0, blue triangles) and Day 15 (black asterisks), respectively. The absorption spectra for these cases show that the levels of HbO_2 increased in both subjects across time, but the relative increase for the complete responder was more pronounced. The scattering coefficient showed increases for both animals in time.

Fig. 4a-4d show the mean fractional longitudinal trends for the tumor volume, SO_2, THb and $<\mu'_s>$ across each group, respectively.

The fractional increase for each endpoint at each any point in time for each animal was defined as the ratio of the endpoint at that time divided by the value of the endpoint at baseline for each animal. The error-bars on the figures represent the standard error computed for the fractional increase across all animals in the group, at each point. In these figures, the red circles indicates the trends in the control, the black triangles the PR group, and the blue squares the CR group.

The fractional increase in SO_2 shows separation between all the groups, with the CR group values showing the steepest increase in SO_2 across time. These trends are similar to those we observed in a previous report [5].

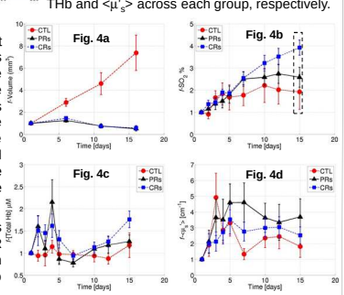
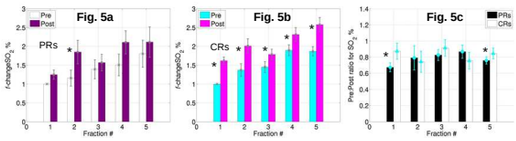


Fig. 5a-5c show the mean fractional increase (relative to baseline) in SO_2 values measured pre- and post-irradiation for the PR (Fig 5a) and CR (Fig 5b) groups. Fig 5c compare the ratio of the pre/post SO2 for these groups.



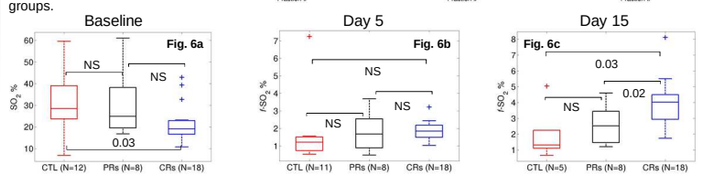


Fig. 6a-6c compare the differences in optical SO_2 values at baseline, day 5 and day 15 across the three groups. Significant differences in the fractional increase of SO_2 were noted between the CR and other groups by day 15. However, there were no differences in oxygenation between the PR and CR groups before then.

HUMAN STUDIES

- 8 Patients with confirmed HNSCC lesions that were accessible via the oral cavity were consented
- CT based treatment was planned in customized immobilization devices with IV contrast
- Planned Target Volume (PTV) dose for each lesion was 70 Gy (delivered in 2Gy daily fractions)
- Optical probe guided to lesion surface by the oncologist and was held in place by patient
- 1-3 locations were scanned to obtain diffuse reflectance from different sites at each time-point

Clinical measurement schedule

F/U indicates follow-up measurements obtained 2-3 months post treatment.

	Baseline	Week 1	Week 2	Week 3	Week 4	F/U
Optical	x	x	x	x	x	x
RT	x x x x x	x x x x x	x x x x x	x x x x x	x x x x x	

Patient	Treatment	TNM Stage	Treatment outcome (12-16 months)
P1	CRT	T3N0M0	No evidence of cancer
P2	CRT	T2N1M0	No evidence of cancer
P3	XRT	T2N1M0	No evidence of cancer
P4	CRT	T3N2bM0	No evidence of cancer
P5	CRT	T2N2aM0	Regional persistence*
P6	CRT	T4aN3M0	No evidence of cancer
P7	CRT	T3N2cM0	Regional persistence#
P8	XRT	T4N0M0	Local regression*#

TABLE 2: Patient outcomes in Human Studies

*: Distant Metastasis, # Patient deceased;
CRT= chemo-radiation therapy; XRT = X-radiation therapy

Table 2 shows the treatment plan, stage and outcome for the patients recruited in the study. Two of the eight patients were noted to have regional persistent disease and were classified as non-responders (NRs) while the 6 other patients were classified as complete responders (CRs).

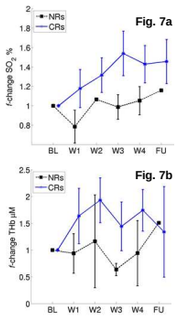


Fig. 7a and **7b** show the longitudinal trends for fractional change in tumor oxygen SO_2 and total hemoglobin THb for the non-responders (black asterisks) and responders (blue squares), respectively. As in the animal study, the error bars represent standard errors in the optical endpoints computed across patients, at each time point. It is seen that the CR group of patients showed increases in fractional tumor oxygenation changes across time, while these changes were not as high in the NR group. However, given the small sample size of the study, no statistically significant differences were found.

Comparison of Fig 7a to Fig. 4b shows that the temporal trends for changes in fractional SO_2 between responders and non-responders was very similar in the animal and human study measurements.

SUMMARY AND CONCLUSIONS

- Longitudinal optical measurements obtained on HNC lesions during radiation therapy were quantified to extract vascular oxygenation and blood volume in mice and human subjects
- The temporal trends for changes in SO2 relative to pre-treatment levels indicated markedly different patterns for subjects that respond to treatment vs. those that did not
- Monitoring vascular oxygenation in solid HNSCC undergoing treatments could provide a non-invasive method to monitor treatment response in individual patients.

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