also plays an important role in affecting tumor response to ionizing radiation [1,2]. However, studying the complex and interdependent relationship between the response of tumor cells and their vasculature in preclinical in vivo models has been a major challenge. To address this, we developed a novel experimental platform to study, non-invasively, the radiobiological response of tumors and their vascular at structural, functional, and cellular levels in solid tumors in vivo.

Methods: Our platform consists of a murine dorsal skinfold window chamber (WC) tumor model, a small animal x-ray microirradiator and multimodal intravital imaging modalities (confocal fluorescence microscopy (CFM) and speckle variance optical coherence tomography (svOCT)). A DsRed-Me180 human cervical carcinoma cell line was implanted in the dorsal skinfold at the time of WC surgery and was grown for 1 week, followed by irradiation (single dose 30 Gy) and multimodal microscopic imaging of multiple tumor components simultaneously in vivo for up to 3 weeks. In addition, laser capture microdissection (LCM) of ex vivo tissues was used to prepare tissues for mRNA microarray to investigate the corresponding RT-induced transcriptomic modifications in irradiated tumors.

Results: CFM was used to provide morphological, structural and functional information about vessels based on blood perfusion of the fluorescent dextran agent (FITC-Dextran), while svOCT was used to provide spatiotemporally correlated maps of patent vascular structures without any contrast agents. Our data showed that a single fraction of 30 Gy to the tumor caused functional disruption in both large vasculature (>70 μm) and capillary (<40 μm) sized microvasculature, while leaving most of the large vasculature structurally intact. These hemodynamic effects were most prominent between days 8-14 following treatment (Figure 1). Moreover, we observed an increase in microvascular density after day 14 to a level significantly higher than pre-treatment, possibly indicating RT-induced neovascularization. The functional disruption of irradiated vessels could be attributed to RT-induced platelet thrombosis, which was observed as early as 1h after RT. We also observed a decrease in the coverage of perivascular cells around tumor vasculature 4 to 8 days after RT. Moreover, these RT-induced structural, functional and cellular changes in the microvasculature in vivo were accompanied by alterations in gene expression. Briefly, 4 days after RT, vascular-related genes (Vegfa, Mmp9 and Mmp2) were up-regulated in the irradiated tumor, while smooth muscle and pericyte-related genes (Des, Myh4, Ttn and Mybpc2) were down-regulated, which correlated well with our in vivo imaging data. A number of eukaryotic initiation factors were also down-regulated, suggesting translational machinery dysregulation preceding tumor cell apoptosis.

Conclusions: Our multimodal platform overcomes previous technical limitations in preclinical studies of radiobiological response: i) the microirradiator enabled precise focal treatment of small tumors in the dorsal skinfold WC with a controlled dose, and ii) the WC model allowed for simultaneous monitoring of multiple tumor components with single-cell resolution using CFM, and for visualization of the vascular network at the capillary level using svOCT. The major advantage of our approach over conventional xenograft models is the ability to study spatio-temporally specific radiation response of interdependent components within solid tumor longitudinally at high resolution in vivo. Lastly, the additional capability of spatially-localized genomic analysis was used to correlate genetic changes with in vivo optical imaging data obtained longitudinally, allowing us to gain new insights into possible mechanisms of RT-induced modifications. Thus, our results demonstrate the capability of this new preclinical experimental platform to enable quantitative, high-resolution and multiparametric intravital optical imaging of complex and dynamic radiobiological changes occurring within the living tumor systems.

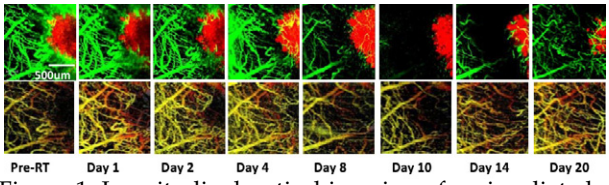

Figure 1: Longitudinal optical imaging of an irradiated tumor using fluorescence confocal microscopy (top: FITC-Dextran in green, DsRed-Me180 in red) and depth-encoded svOCT (bottom), demonstrating dynamic functional and structural changes in tumor vasculature following treatment with single dose 30 Gy RT.

1.Garcia-Barros M. et al., Tumor response to radiotherapy regulated by endothelial cell apoptosis. Science 2003; 300(5622): 1155-9.

2.Fuks, Z. and R. Kolesnick, Engaging the vascular component of the tumor response. Cancer Cell 2005;8(2): 89-91.

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DOES QUALITY OF RADIOTHERAPY PREDICT OUTCOMES OF MULTICENTRE CLINICAL TRIALS? THE EORTC RADIATION ONCOLOGY GROUP EXPERIENCE.

A. Fairchild, L. Collette, C. Hurkmans, B. Baumert, D. Weber, A. Gulyban, P. Poortmans.

Introduction: The EORTC dummy run (DR) was implemented in 1987 to address early signs that institutions could not meet minimum technical requirements for Radiation Oncology Group (ROG) trials. After central review of a DR (practice) case, reviewer comments are sent to local principal investigators (PIs). This feedback is ideally applied to subsequent protocol patients' RT planning. The goal of the individual case review (ICR), originating in 1989, is systematic assessment of treatment received to evaluate actual protocol compliance. Since 1991, DR and ICR results have been published for trials on cancers of the breast, prostate, brain and head and neck. Our objective was to review EORTC radiotherapy (RT) quality assurance (QA) data to investigate whether completion of a DR increases success rates for other QA procedures or impacts patient outcomes.

Methods: EORTC protocols closed to recruitment were reviewed for inclusion of a DR procedure. Candidate studies were randomized phase III studies in which the ROG managed trial QART. Data was collected, evaluated, translated, reformatted, and collated into databases. If a DR case grade was either acceptable or

minor deviation (at first submission), this was sufficient for authorization to participate in a trial and was considered a success (at first attempt). A DR was classified as a failure if issues were found which required correction and re-submission. Trials with a DR procedure were reviewed for ICR results and mature clinical outcomes. Similar to DR, ICR datasets were graded overall as acceptable, major or minor violation. Fisher's exact test was used to characterize potential correlations and the Mantel-Haenszel statistic provided estimates of pooled odds ratios (OR).

Results: 25 closed ROG protocols implemented a DR. Raw data were available in 12, of which success at first attempt could be reconstructed in six, and ICR results were known for three (Table 1). The proportion of institutions successful at first DR attempt varied from 5.6% to 68.8%. Of those who previously participated in a DR, between 8.3-76.9% were successful on the first attempt in the current trial. There was a significant correlation between past DR participation and success at first attempt for the 22991 trial (p=0.04); this was a trend for 10981 (p=0.06), and for the remainder, there was no correlation. Institutions were 3.2 times more likely to be successful at first DR attempt if they had previously participated in this procedure (95% CI 1.73-5.93; p=0.0002). Of all patients reviewed during an ICR, the proportion from institutions successful at DR first attempt was on average 62.0%. Of these, approximately half (52.3%) had an overall ICR grade of acceptable. There was a significant correlation for the 22991 trial only (p=0.03). Pooled OR was 1.69 (95% CI 0.97-2.95; p=0.06). Repeating this analysis including patients receiving an ICR grade of either acceptable or minor deviation did not change the conclusions. Mature clinical outcomes were available for the 22911 trial only; median follow-up for all patients was 10.6 years. 389 patients from 26/36 participating institutions were included in this analysis. For patients irradiated by a site which participated in the DR, 5 year progression-free survival (PFS) was 81.6% (95%CI 76.7-85.4%), compared to 79.8% (95%CI 69.1-87.1%) for patients from non-participating sites. This difference was, however, not significant on either univariate (HR 0.91; 95%CI 0.65-1.45; p=0.88) or multivariate analysis (HR 1.09; 95%CI 0.70-1.68; p=0.70) adjusted for statistically significant patient and disease factors (age, nerve-sparing surgical procedure, post-operative PSA, seminal vesicle invasion).

Conclusions: In this review of two decades of data from the EORTC ROG, institutions which previously participated in a DR were significantly more likely to be successful at subsequent first DR attempts. With the exception of the 22991 prostate trial, this correlation was not significant for any specific trial, likely due to small sample sizes. For the 22991 trial, sites which were successful at first DR attempt were also significantly more likely to deliver protocol-compliant RT, based on results of the ICR. In the 22911 trial, however, a significant effect of DR participation on 5yr PFS could not be demonstrated.

Table 1. Summary of trials included in analysis. *Data available on DR success at first attempt. ¶ICR results available. §Trial not centrally activated or did not complete planned accrual. Abbreviations: chemo – chemotherapy; CUP – carcinoma of unknown primary; H&N – head and neck; LN - lymph node; postop - postoperative; RT - radiotherapy.

Trial	DR Completed	Trial Randomization
Prostate		
22863	1991-1992	Pelvic RT vs pelvic RT + hormone therapy
22911	1998-2000	Immediate postop RT vs RT at local relapse
22961	2001-2003	RT + 6 months concurrent hormone therapy +/- 2.5 years adjuvant hormone therapy
22991*¶	2001-2006	RT +/- concurrent hormone therapy in early stage disease
Breast		
22881	1989-1995	Breast RT boost vs no boost post-segmental resection
22922*¶	1996-2002	Internal mammary LN RT vs no internal mammary RT
10981*	2001-2009	Axilla RT vs surgery after positive sentinel LN procedure
H&N		
22931	1994-1997	Postoperative chemoRT vs RT alone
24001*§	2003-2004	Extensive H&N RT vs ipsilateral neck RT only for CUP
22071*§	2010-2011	Postop chemoRT +/- concurrent anti-EGFR antibody
Brain		
22972§	1999-2001	Stereotactic boost vs no boost for high grade glioma
22033*¶	2006-2010	Primary chemotherapy vs RT for low grade glioma

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DOES QUALITY OF RADIOTHERAPY PREDICT OUTCOMES OF MULTICENTRE COOPERATIVE GROUP TRIALS?
A LITERATURE REVIEW.

A Fairchild
Cross Cancer Institute

Introduction: Central review of radiotherapy (RT) delivery within multicentre clinical trials was initiated in the early 1970's in the USA. Early quality assurance (QA) performed by the CALGB and other clinical trial groups revealed non-uniformity of treatment strategies. Aside from the suggestion of increased patient evaluability with central QA office intervention, initial publications often focused on metrics related to process, logistics and timing. The objective of this work was to review available evidence for correlation of RT quality with clinical outcomes within multicentre cooperative group clinical trials.

Methods: An OVID medline search was performed restricted to the English language, but with no restriction on date of publication. Candidate multicentre studies accrued adults only, were led by any cooperative group, and described central subjective +/- objective assessment of RT protocol compliance (quality). The QA publications and, where necessary, other papers describing trial clinical results were evaluated. Data abstracted included method of central