Final Protocol

Title	**Randomised double-blind controlled phase III trial of hyperbaric oxygen therapy in patients suffering long-term adverse effects of radiotherapy for pelvic cancer**
Short Title	**HOT II**
Protocol Reference:	**CCR 3086 / REC 08/H0903/40**
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Sponsor:	**The Royal Marsden NHS Foundation Trust and The Institute of Cancer Research**
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Revision Chronology	Effective Date	Reason for Change
Vs 3, 16.09.08	23.09.08	Original Ethics Approval
Vs 4, 04.11.09	20.01.09	Change to one CI, additions to HEQ, addition of site
Vs 5, 20.01.09	05.03.09	Change of sponsor
Vs 6, 09.07.09	01.09.09	Addition of CTC, side effects and amended eligibility criteria
Vs 7, 21.01.10	13.04.10	PIS and consent form separated from protocol. Eligibility assessment forms and clinical report forms listed but separated from protocol; inclusion of assessment over the telephone followed by postal consent; review of section 7 following internal audit of Research Governance; list of treatment centres replaced with reference to current PIS
Vs 8, 05.07.10	23.08.10	Change of PI & addition of two further treatment sites
Vs 9, 05.10.10	10.12.10	Change of Trial Coordinator + minor changes to sections on Expected Adverse Events (5.7.3) and Patient Confidentiality (8.3)
Vs 10, 15.12.10	17.02.11	Change of PI + minor administrative changes to sections 4.3, 5.1 and 5.2
Vs 11, 24.03.11	14.04.11	Change of identifiers on CRFs in line with GCP (appendices 1-5) + minor administrative changes to pages 5, 6, 17 (IBDQ at 2 weeks post HBO omitted from protocol in error)
Vs 12, 05.05.11	01.08.11	Addition of treatment centre in Poole
Vs 13, 05.08.11	14.10.11	Modification of pre-entry eligibility assessments (4.3). Change of End of Study definition (7.8).
Vs 14, 27.02.12	11.04.12	Addition of second primary endpoint (5.6.1) and subsequent statistical considerations (6). Change to inclusion criteria (4.1).
Vs 14.1, 21.06.12	19.06.12	Admin error in amended sample size calculation (6.3).
Vs 15, 14.02.13		Change of definition of End of Study (7.8) and reference to DSUR (5.7.5)

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SUMMARY

Aim
To test the clinical benefits of hyperbaric oxygen therapy in reducing dysfunction in patients developing iatrogenic gastrointestinal symptoms as a result of previous radical pelvic radiotherapy for cancer, which was completed at least one year previously.

Trial design
Randomised double-blind controlled phase III trial.

Eligibility
Inclusion criteria
i) Age over 18 years.
ii) Past history of a malignant pelvic neoplasm (T1-4 N0-2 M0), including carcinoma of the rectum, prostate, testis, bladder, uterine cervix, uterine corpus, vagina, vulva and ovary.
iii) A minimum 12 months follow-up post-radiotherapy (36 months for patients with past history of stage T4 and/or N2 disease).
iv) No evidence of cancer recurrence.
v) Gastrointestinal symptoms attributable to prior radiotherapy: grade 2 or higher in any LENT SOMA category, or grade 1 with difficult intermittent symptoms.
vi) Symptoms are not relieved by appropriate life-style advice and medication over a 3-month period.
vii) Physical and psychological fitness for HBO therapy.
viii) Written informed consent and availability for follow up.

Exclusion criteria
i) Surgery for rectal cancer.
ii) Prior hyperbaric oxygen therapy (excluding treatment for decompression illness).
iii) Prior treatment with even a single dose of bleomycin.
iv) Claustrophobia.
v) Epilepsy.
vi) Chronic obstructive airways disease; bullous lung disease, acute or chronic pulmonary infection; uncontrolled asthma, untreated pneumothorax.
vii) Previous middle/inner ear operations (except grommets and similar procedures) &/or irremediable inability to equalise middle ear pressure.
viii) Contra-indication or other inability to undergo magnetic resonance imaging, if required to rule out malignancy.

Randomisation
75 patients eligible for the study will be randomised by a hyperbaric technician using a 2:1 ratio of treatment:control via a telephone call to the Clinical Trials and Statistics Unit (ICR-CTSU) at the Institute of Cancer Research, Sutton, Surrey. Contact 020 8643 7150 between 9am and 5pm, weekdays.

Hyperbaric oxygen therapy
Treatment group
Patients will be compressed to 2.4 ATA in a hyperbaric chamber and will breathe 100% oxygen while at pressure following Royal Navy Therapeutic Table 66 (RNTT 66). The total time at 2.4 ATA will be 90 minutes. Each participant will receive a total of 40 pressure exposures (five days per week for eight weeks).

Control group
Patients will be compressed to 1.3 ATA in a hyperbaric chamber and will breathe 21% oxygen (air) while at pressure. The total time at 1.3 ATA will be 90 minutes. Each participant will receive a total of 40 pressure exposures (five days per week for eight weeks).

Primary clinical endpoints
i) Overall gastrointestinal symptoms score using the modified Inflammatory Bowel Disease Questionnaire (IBDQ), using a 3% p-value.
ii) Change in rectal IBDQ bleeding score between the two groups, using a 2% p-value.

Secondary clinical endpoints
i) Physician assessment of bowel dysfunction using LENT SOMA scales of radiation injury.
ii) Patient self-assessments using EORTC QLQ-C30 and Defaecation Problem Subscale of QLQ-CR38.
iii) Photographic images of rectal mucosa taken via flexible sigmoidoscopy.
iv) Physician assessment of rectal dysfunction based on the modified CTCAE grading.

Translational endpoints
i) Rectal biopsies: Increase in blood vessel density will be investigated. This component of the research will use immunohistochemistry on tissue sections. Changes will also be investigated in proteins involved in extracellular matrix metabolism, including fibrogenic cytokines (e.g. CTGF, $TGF\beta_1$), collagen synthesis (e.g. PINP, PIIINP, prolyl-4-hydroxylase) and metalloproteinases (e.g. MMP-I).

Follow up
Clinical post-treatment assessments will be performed at The Royal Marsden, London, within 14 days of completing the treatment and at 12 months after the start of hyperbaric oxygen (HBO) therapy. For patients unable to attend The Royal Marsden, assessments using the LENT SOMA and CTCAE forms will be carried out by a Clinical Nurse Practitioner via a telephone interview. All patients will be asked to complete self-assessment questionnaires, including Health Economics, IBDQ, EORTC QLQ-C30 and CR38, at 3, 6, 9, and 12 months after the start of HBO therapy (+ IBDQ only at 2 weeks after completion of HBO).

Treatment Locations
Please see current Patient Information Sheet for list of collaborating hyperbaric medicine centres.

To recommend a patient for eligibility assessments, please contact us by mail, telephone or fax
Professor John Yarnold, Academic Radiotherapy Dept
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Tel: 020 8661 3273. FAX: 020 8661 3107. Email grace.sharp@icr.ac.uk

The following information will be required at referral:
- Referring clinician's full name and address
- Patient's full name, address, date of birth and GP details
- Tumour site, radiotherapy details
- Details of radiotherapy-induced complication
- Details of previous investigations and management

This trial will be conducted in compliance with the protocol, GCP and regulatory requirements.

HOT II - SCHEDULE OF EVENTS

Procedure	Pre-entry eligibility assessments (local gastro-enterologist)	Pre-entry eligibility assessment and randomisation (HMUs)	Assessments prior to randomisation and consent (RMH)	Treatment (weeks) (HMUs)								Follow up 0-2 weeks post end of HBO therapy (RMH)	Follow-up 3-9 months post start of HBO therapy (by post)			Follow up 12 months post start of HBO therapy (RMH)
				1	2	3	4	5	6	7	8		3	6	9	
Assessment and treatment (4.3)	√															
CTCAE Grading	√		√									√				√
Consent procedure (4.3)			√													
LENT SOMA assessment (app. 2)	√		√									√				√
Patient self-assessment questionnaires (app. 1 & 4)			√									√ IBDQ only	√	√	√	√
Rectal photographs (5.1)*			√									√				√
Rectal biopsies (5.1)*			√									√				√
HBO clinical assessment & consent to treatment (4.3)		√														
Randomisation by HMUs (5.2)		√														
HBO therapy (5.4.1)				√	√	√	√	√	√	√	√					
Health Economic questionnaire (app. 5)			√ post randomisation, but pre treatment										√	√	√	√

* For patients attending The Royal Marsden only.

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Assessment centre:	The Royal Marsden, Fulham Road, London SW3 5JJ
Treatment centres:	Please see current Patient Information Sheet for full list of treatment centres

SIGNATURE OF APPROVAL

Function	Name	Signature	Date
Chief Investigator	**Prof John Yarnold**		**14.02.2013**

1 INTRODUCTION

1.1 Radiotherapy has a proven role in the curative treatment of pelvic malignancies

Level I evidence supports the use of radiotherapy to improve local tumour control and long-term survival of patients with carcinoma of the rectum, rectosigmoid [3, 28] and cervix [81]. There is also level I evidence that radiotherapy contributes to cure in patients with carcinoma of the anus [2]. At these primary tumour sites, radiotherapy combined with surgery and/or cytotoxic therapy is standard treatment for a high proportion of patients. Radiotherapy alone or combined with cytotoxic therapy and/or surgery is also regarded as standard treatment at a number of other primary tumour sites in the pelvis, including bladder [80], caecum [139], prostate [98], endometrium [97], vagina [123] and vulva [124]. The number of patients diagnosed with pelvic malignancy in the UK is approximately 82,500 per year [1]. Of these approximately 12,000 undergo pelvic radiotherapy annually. Across the developed world, the total number of patients being treated annually has been estimated to range from 150,000 to 300,000 [8, 45]. In the United States, there are estimated to be 2.5 million long-term survivors following pelvic irradiation.

1.2 Radiation-induced gastrointestinal injury causes severe functional disability

Treatment-induced complications are an inevitable consequence of increased local tumour control rates and overall survival, but chronic iatrogenic symptoms exact a high price terms of impaired quality of life [39]. Virtually every patient undergoing radiotherapy to the abdomen, pelvis, or rectum develops symptoms of acute bowel toxicity, but these are usually not significant in the long-term [55, 91]. The development of late-onset bowel toxicity is related to radiation dose, volume, time and fractionation [16, 22]. It is also influenced by other treatment and patient-related factors which are poorly characterised, partly due to a lack of uniformity in methodologies used to define significant late toxicity [8]. Once the acute mucosal reactions have settled, there are broadly speaking four long-term outcomes for patients in terms of bowel function: 10-20% of patients have no long-term sequelae; 30–40% notice some change in bowel habit without any effect on quality of life; in at least 30% of patients, change in bowel function interferes significantly with daily activities and long-term quality of life; finally, a small minority develops life-threatening changes, including transfusion-dependent bleeding, fistula formation, bowel obstruction and secondary malignancy. The incidence of such severe problems is not known precisely, but it is estimated that 4-10% of patients are affected over the first 5-10 years [27, 42, 93] and 15-20% over 20 years [30]. Although some symptoms may improve with time [90], most chronic syndromes associated with radiotherapy are progressive and irreversible [127]. As the number of UK patients undergoing successful pelvic radiotherapy increases, the morbidity of chronic radiation bowel toxicity becomes more common [91].

The frequency of significant bowel problems cited above is higher than many clinicians recognise. This is because prospective studies using comprehensive, validated methodology concentrating on bowel toxicity with adequate follow up are few in number. Prospective follow up of 77% of 5-year survivors in the Swedish Rectal Cancer Trial testing preoperative high-dose radiotherapy revealed that 30% of the irradiated group had significant impairment of social life due to bowel dysfunction, compared with 10% of the surgery-alone group [23]. In another multicentre study which looked at the role of glutamine in protecting against acute radiation toxicity, in which about two-thirds of patients had prostate cancer and most of the rest gynaecological cancer, 2-year follow up was available in 57%. Of these, 12% reported bowel problems moderately affecting daily activity [62]. In a third prospective study, a small subset of surviving patients who had undergone adjuvant radiotherapy following surgery for

rectal cancer, were compared to a subgroup of patients who had been randomised within the trial to surgery alone. Bowel frequency (80% vs. 23%), loose or liquid stool (60% vs. 23%), fecal incontinence (60% vs. 8%) and need to wear a pad more often (47% vs. 0%) were all significantly more frequent in patients allocated radiotherapy [67].

Physician assessments appear to under-report morbidity. For example, the frequency and severity of gastrointestinal toxicity 18 months post-radiotherapy in a series of prostate radiotherapy trials was 29-45% grade 1, 5-14% grade 2, 0.6-3% grade 3 and 0-1% grade 4 [18, 19, 25, 63, 76, 145]. However, toxicity was a secondary endpoint and was measured using crude Radiation Therapy Oncology Group (RTOG) external assessments [55, 92, 144]. A rather different and more sobering picture is obtained from retrospective studies based on patient self-reporting. These studies reveal that patients often do not report toxicity for a variety of reasons [8], and that new bowel symptoms are common and have the greatest impact on quality of life [10]. These studies suggest that at least 30% of bladder and gynaecological patients and 20% of all patients treated for prostate cancer, have 'gastrointestinal symptoms causing moderate or severe distress' as a result of their radiotherapy [6, 15, 34, 48]. Two other studies using questionnaires proposed for use in the HOT II trial found that 50% patients are left with long-term chronic gastrointestinal side effects interfering with daily activity [37, 92]. Results in the rectal patients were similar to those described in an earlier detailed case controlled study [60].

1.3 Chronic symptoms and their current management

Iatrogenic symptoms developing after pelvic radiotherapy include; bleeding per rectum, bloating, constipation, cramps, diarrhoea, faecal incontinence, flatulence, frequency of defaecation, inability to differentiate solid from liquid stool or gas, inability to differentiate need to defaecate from need to pass urine, mucus discharge, nausea, abdominal, rectal, anal or perineal pain, perineal irritation, steatorrhoea, tenesmus, urgency, need for nocturnal defaecation and weight loss. These symptoms in turn lead to major psychological, financial, sexual and social problems.

The management of chronic radiation-induced bowel symptomatology is inadequate. There is seldom a systematic attempt to identify affected patients, few gastroenterologists see many patients and few feel confident to investigate and manage them [8]. Several studies have shown that specific symptoms developing after pelvic radiotherapy do not predict the underlying cause for those symptoms [7, 79, 102, 135, 140], yet few patients undergo systematic investigation when they do develop symptoms. If this is carried out, it shows that the majority of patients have more than one cause for their symptoms [8]. This may be the reason why the very few trials of treatment have been so disappointing, in that the underlying problems being treated have been so poorly defined [26]. The main pharmacological remedy is anti-diarrhoea medicine prescribed to slow bowel transit time and to increase stool consistency. Medication was needed by more than 50% of patients treated with chemo-radiotherapy for rectal cancer in the study by Kollmorgen and colleagues [60]. Surgical management is reserved for severe injuries [117]. This carries a significant rate of complications, including an operative mortality of 10-21% and a 6-36% incidence of anastomotic dehiscence [65, 141]. Even after successful surgery, symptoms persist in a significant proportion of patients [141].

1.4 Mechanisms of tissue atrophy and fibrosis after radiotherapy

Symptoms develop due to primary loss (atrophy) of the absorptive surface and specialised parenchymal cells causing lactose intolerance or bile salt malabsorption, primary or secondary

damage to the gastrointestinal autonomic plexus causing altered bowel motility and injury to muscles and sphincters. Fibrosis is a prominent component of late radiotherapy change in the blood vessels, gastrointestinal tract and urogenital system [107], where it causes progressive tissue stiffness and contraction of the bowel and bladder [22, 68, 96]. Contraction of fibrous tissue causes strictures in hollow viscera such as the gastrointestinal tract and ureter, precipitating obstructive complications.

Chronic tissue atrophy and fibrosis after radiotherapy are partly secondary responses to radiation-induced vascular injury [50, 51]. We hypothesise that a fibrogenic response to hypoxia, mediated via a range of hypoxia-regulated genes, contributes to tissue scarring, a mechanism increasingly recognised in other chronic fibrotic states. A close association has been noted between interstitial fibrosis and microvascular damage in progressive renal disease [89]. In this example, a sequence of events involving a heme-protein sensor is postulated, similar to the sensor involved in erythropoietin (*epo*) gene regulation. This activates signal pathways responsible for upregulation of transcription factors, including hypoxia-inducible factor-1α (HIF-1α) [82, 100, 112, 138]. Several studies report upregulated *coll-i* gene expression and increased production of collagen-α1(i) in response to low oxygen tension [31, 119]. Downregulation of collagenase (MMP-1) and upregulation of metalloproteinase (TIMP-1) at mRNA and protein levels have also been reported. A role for these mechanisms in renal disease is supported by the interaction demonstrated between HIF-1α and hypoxia response elements in transient transfection experiments using a TIMP-1 promoter CAT reporter construct [89]. The potential implications for radiation-induced fibrosis are that progressive capillary injury may deregulate extracellular matrix metabolism via modulation of hypoxia-regulated genes. The clinical implications are that restoration of normal tissue oxygen tension via induction of angiogenesis may halt or reverse these processes.

1.5 Some major radiotherapy adverse effects are reversible

Hyperbaric oxygen (HBO) therapy currently provides the strongest clinical evidence that radiotherapy morbidity can be modified, with two comparative studies reported in patients with heavily damaged tissues. The first study tested perioperative hyperbaric oxygen against conventional penicillin cover in a randomised study of 74 patients requiring dental extraction following radical mandibular irradiation [75]. Only 5.4% of the patients in the hyperbaric oxygen group compared to 30% of the patients in the penicillin-treated group experienced failure of wound healing 6 months after surgery ($p<0.05$). In 160 patients treated by pre and post surgery hyperbaric oxygen or standard postoperative care following major soft tissue surgery for radiotherapy injury, four-fold reductions in wound dehiscence, infection and delayed wound healing were seen in the hyperbaric oxygen treated group ($p<0.01$) [72]. No adverse effects of hyperbaric oxygen were reported in either of these studies.

Level II evidence of effect is available supporting the efficacy of HBO for the treatment of severe radiation-induced haemorrhagic cystitis refractory to conventional measures. The largest study describes 40 patients treated for 20 sessions of HBO [17]. Thirty-seven of the 40 patients showed marked and durable (up to 5 years) reductions in bleeding frequency, including need for blood transfusions, and no adverse effects were reported. A consecutive series of 20 patients treated between1989-1992 reported improvement of haematuria in 90% of cases [64]. Similar experiences based on smaller patient numbers are available [56, 64, 88, 111, 137]. No adverse effects of HBO were reported in any of these studies.

The efficacy of HBO in the management of radiation-induced bowel injury, including proctitis, is reported in a series of case studies and small series reporting improvement in pain, tenesmus, bleeding, diarrhoea and rectal ulceration [13, 24, 43, 57, 83, 84, 87]. More significantly, there are a number of retrospective studies describing complete or partial healing of rectal symptoms in patients resistant to conventional therapy for severe injuries. One of the largest retrospective studies describes 36 patients, including 9 with chronic necrotic wounds, 19 with chronic rectal bleeding and 9 with chronic severe diarrhoea [40]. Severity of symptoms according to the LENT SOMA scale [95, 108] was grade 4 in 8 patients, grade 3 in 16, grade 2 in 12 and grade 1 in one patient. After 67 (range 12-198) HBO sessions, the authors reported 9 complete responses, 12 partial responses and 11 non-responses (in addition, one patient died from radiation injury, two from cancer and one from liver cirrhosis). In a study of 38 patients treated with HBO for chronic, uncontrolled rectal bleeding, treatment resulted in an improvement in 61% of cases [4]. A third retrospective study of 18 patients reported complete or partial responses after a mean number of 24 sessions of HBO (range 12-40) in 7/17 cases rectal bleeding, 2/4 pain syndromes, 3/4 cases of faecal incontinence and 4/8 diarrhoeal syndromes [142]. These experiences are broadly repeated in other studies, including a cohort of 14 patients, 11 of whom presented with rectal bleeding, 5 with diarrhoea and 5 with tenesmus or colic [136]. After a mean number of 40 HBO sessions (range 20-72), 9 patients were completely healed, 3 patients improved substantially and 2 were non-responders.

The most important initiative, however, is an international double-blind placebo-controlled randomised clinical trial (Hyperbaric Oxygen Radiation Tissue Injury Study, HORTIS), incorporating a cross-over design, conducted by the Baromedical Research Foundation in South Carolina, USA. It has eight components, including seven evaluating HBO for established radio-necrosis at varying anatomic sites, including rectum. An interim analysis was undertaken in the 68 patients recruited into the proctitis arm (HORTIS IV) of the study in 2004 [21]. Patients were randomised to receive either oxygen at 2.0 ATA or air at 1.0 ATA. The two groups were compared for pre- and post-exposure changes in both clinical findings and SOMA LENT scale. Of the 30 patients in the treatment group, 16 (53.3%) were healed or had significant improvement; only 6 of 27 (22.2%) who received placebo had these outcomes, none of whom were healed. Logistic regression indicates significance ($p=0.0146$), with OR=4.00 (1.25, 12.72). Change in LENT scale was also significant between the two groups ($p=0.0015$), with a larger change in the treatment group than in the placebo group (4.60 vs. 0.65). A potential limitation of this valuable study is the cross-over design implemented 30 days after the end of first treatment. The trial effectively becomes an observational study after early cross-over with unblinded response evaluation by clinicians beyond this time-point.

Although limited in some respects, these reports are encouraging, in that chronic haemorrhage from the bladder or rectum can be reliably scored in a semi-quantitative manner, including the number of patients freed from the requirement for repeated blood transfusions over the months and years post-therapy. Taken together, the evidence lends strong support to the hypothesis that HBO is an effective treatment in a substantial proportion, if not a majority, of patients suffering complications following radiotherapy for pelvic cancer. However, apart from HORTIS, the data are derived from retrospective studies, and are characterised by a lack of consistency with respect to the scoring of symptoms and response criteria. Until well-designed randomised clinical trials are undertaken, patients will continue to be referred to hyperbaric facilities on an ad hoc basis, and effective treatment for a common and major morbidity may continue unrecognised and under-utilised.

1.6 Hyperbaric oxygen stimulates neoangiogenesis in ischaemic tissues

Irradiated tissues are characterised by hypocellularity, fibrosis, low oxygen tensions and shallow oxygen gradients [73]. At the pathological level, irradiated tissues have a capillary density 20-30% that of unirradiated areas [73, 74]. During hyperbaric oxygen (HBO) exposure, there is a 7 to 10 fold rise in oxygen tension and a steep oxygen gradient across the damaged zone. Steep oxygen gradients appear to stimulate angiogenesis, fibroplasia and other features of tissue restructuring in animal models [58, 59, 115]. In the early stage of HBO therapy, capillary budding and cellular collagen synthesis occur in animal systems [74]. This is followed by a rapid rise in the number of lumenised capillaries to 75-80% that of normal tissues. As new capillaries form, hypoxia is reversed and the oxygen gradient is lost, with down-regulation of angiogenesis. No increase in vascularity is seen in adjacent undamaged tissue. Further increases in oxygen tension abolish these effects and are toxic. How these physiological responses in animal models relate to functional improvement reported in patients is unclear. It is not difficult to imagine how a renewed capillary network restores the integrity of the rectal epithelium, and reduces rectal bleeding from telangiectatic vessels. It is also possible that reduced capillary leakage leads indirectly to improved rectal compliance via a reduction of oedema in the rectal wall and ischio-rectal fossa. It is conceivable that restoration of tissue perfusion reverses a chronic stimulus of fibrogenesis perpetuated by radiation-induced vasculitis and hypoxia. Nevertheless, the physiological mechanisms that mediate vascular proliferation and tissue healing are poorly understood, much less the molecular mechanisms, see Appendix 6.

1.7 Associated risks of hyperbaric oxygen

Complications of hyperbaric oxygen (HBO) include barotrauma (ear or sinus trauma, tympanic membrane rupture, lung rupture, pneumothorax and gas embolism), oxygen toxicity (seizures and pulmonary toxic reactions), reversible visual changes, cataract and claustrophobia [41]. These are uncommon at pressures contemplated for clinical applications. The theoretical risk that HBO might promote growth of occult foci of pelvic cancer must also be considered. Retrospective data are available in 245 patients with oral cancer following HBO for osteoradionecrosis and surgical reconstruction. These patients were compared to a group treated over a similar period without need of HBO. Stage for stage, fewer recurrences were seen in the HBO group, but the numbers involved were small and the limitations of retrospective studies are well recognised [72]. Carcinomas have been induced by carcinogens in a hamster cheek pouch system [44]. One group was exposed to HBO and compared to an unexposed control group. The tumour growth rates of both groups were similar. In other studies, mouse lung primaries and metastases exposed to HBO showed no acceleration in growth rate in comparison to a control group [86]. No human or animal data are available on pelvic cancer. In conclusion, there are virtually no relevant data relating to cancer promotion by HBO.

2 PROPOSAL

A double-blind randomised controlled phase III trial of hyperbaric oxygen therapy in patients suffering long-term gastrointestinal adverse effects of pelvic radiotherapy.

3 AIM

To test the clinical benefits of hyperbaric oxygen therapy in reducing dysfunction in patients developing iatrogenic gastrointestinal symptoms as a result of previous radical pelvic radiotherapy for cancer, which was completed at least one year previously.

4 PATIENTS

Eligibility will be confirmed by a specialist in hyperbaric medicine and a consultant in gastroenterology with special expertise in the management of late radiation-induced bowel injury.

4.1 Specific inclusion criteria

i) Age over 18 years.
ii) Past history of a malignant pelvic neoplasm (T1-4 N0-2 M0), including carcinoma of the rectum, prostate, testis, bladder, uterine cervix, uterine corpus, vagina, vulva and ovary.
iii) A minimum 12 months follow-up post-radiotherapy (36 months for patients with past history of stage T4 and/or N2 disease).
iv) No evidence of cancer recurrence.
v) Gastrointestinal symptoms attributable to prior radiotherapy: grade 2 or higher in any LENT SOMA category, or grade 1 with difficult intermittent symptoms.
vi) Symptoms are not relieved by appropriate life-style advice and medication over a 3-month period.
vii) Physical and psychological fitness for HBO therapy.
viii) Written informed consent and availability for follow up.

4.2 Specific exclusion criteria

i) Surgery for rectal cancer.
ii) Prior hyperbaric oxygen therapy (excluding treatment for decompression illness).
iii) Prior treatment with even a single dose of bleomycin.
iv) Claustrophobia.
v) Epilepsy.
vi) Chronic obstructive airways disease; bullous lung disease, acute or chronic pulmonary infection; uncontrolled asthma, untreated pneumothorax.
vii) Previous middle/inner ear operations (except grommets and similar procedures) &/or irremediable inability to equalise middle ear pressure.
viii) Contra-indication or other inability to undergo magnetic resonance imaging, if required to rule out malignancy.

4.3 Pre-entry eligibility assessments

All patients will undergo assessment of their symptoms by their local gastroenterologist according to a predefined protocol in order to confirm eligibility. Eligibility requires that appropriate life-style advice and a minimum 3-month period of standard medications are unsuccessful in controlling symptoms. After eligibility is confirmed, patients will be reviewed by a medical specialist and research nurse in the Gastrointestinal Late Toxicity Clinic at The Royal Marsden. For patients unable to attend The Royal Marsden, assessments using the LENT SOMA and CTCAE forms will be carried out by a Clinical Nurse Practitioner via a telephone interview. If patients have not undergone colonic/pelvic imaging as deemed appropriate by their clinicians within a reasonable clinical time frame commensurate with their symptoms, this will be requested locally. MRI will be requested if any of the following circumstances: i) anal, rectal, atypical abdominal or back pain as a significant symptom, especially if of more recent onset, ii) endoscopic evidence of anal, rectal or sigmoid stricture, iii) recent change in symptoms a long period after radiotherapy with stable symptoms previously, iv) sudden change of otherwise unexplained symptoms, v) changing urinary symptoms or vi) new onset vaginal bleeding in women. PSA will be requested in patients with a past history of prostate cancer. Patients will have ample time to consider issues surrounding consent, which will be collected at the time of this appointment. Patients will then be required

to attend their nearest hyperbaric medicine unit, where they will be assessed for suitability for hyperbaric oxygen therapy and standard consent for hyperbaric treatment obtained.

5 METHODS

5.1 Pre-treatment assessments

The following must be completed *before randomisation*:

- IBDQ Quality of Life and Bowel Symptom Questionnaire (Appendix 1).
- Physician/Clinical Nurse Practitioner assessments of late radiation-induced side effects using LENT SOMA scales (Appendix 2).
- Physician/Clinical Nurse Practitioner grading of late radiation-induced side effects using the modified Common Terminology Criteria for Adverse Events criteria (CTCAE Appendix 3)
- Patient self-assessments using: EORTC Quality of Life Questionnaires (QLQ-C30 & QLQ-CR38) (Appendix 4).
- For all patients attending The Royal Marsden, the rectum will be photographed in a standardised manner, before any biopsies are taken.
- All patients attending The Royal Marsden will undergo flexible sigmoidoscopy and, with written consent, rectal biopsy (those who have received prostate brachytherapy will not be eligible for biopsy on grounds of safety). Biopsies will be taken in a standardised manner 8 cm from the anal margin*. Mucosa and a minimal amount of submucosa will be sampled under direct vision. The size of the mucosal deficit caused by the biopsies is 2-3 mm and in the patient with radiation proctopathy, this is minimally traumatic. Rectal biopsies (six in total) will be collected via endoscopy before HBO/control therapy in as many volunteers as possible (target is 80% of the whole group). Biopsies will be taken from the posterior third of the rectum. 4/6 samples will be immediately snap frozen in liquid nitrogen for storage. 2/6 samples will be fixed in formalin for future immunohistochemical studies.

  * A pilot study has confirmed that despite the small size of these superficial biopsies, informative samples containing mucosa and some submucosa are easily obtained from irradiated patients using this technique. In further preliminary experiments, we have established that two endoscopically directed biopsies taken from areas of the human rectum showing maximal change from previous radiotherapy are sufficient to obtain adequate amounts of RNA for further analysis.

The Health Economics Questionnaire will be completed post randomisation (as the patients are blind to the allocation) but pre start of treatment.

5.2 Randomisation

Patients will be randomised into the study by a telephone call from the hyperbaric medicine facility where they are due to be treated to the Institute of Cancer Research Clinical Trials & Statistics Unit (ICR-CTSU) on 020 8643 7150. The caller will be given the patient's unique trial identification number (Trial ID) and treatment allocation. The Trial ID should be used on all Case Report Forms and all subsequent correspondence relating to that patient.

The following information will be required *at randomisation*:

- Patient's full name, hospital number, date of birth, NHS number.
- Name of treating hyperbaric centre and person randomising patient.
- Confirmation that patient is eligible for the trial by completion of the eligibility checklist.
- Confirmation that the patient is free of active cancer (MRI report in selected patients).

- Confirmation that the patient has given written informed consent for randomisation on the Consent form.
- Whether or not the patient has given written informed consent for rectal tissue biopsies.
- Confirmation that the pre-treatment assessments listed in section 5.1 have been completed, by completion of Pre-randomisation and Pre-treatment checklists.

5.3 Allocation of treatment

Treatment allocation will be in a 2:1 ratio of HBO Treatment:Control and will be based on computer generated random permuted blocks. Randomisation will be stratified by treating centre and severity (low or high). A fax confirming randomisation will be sent to the hyperbaric medicine unit to enable the allocated study treatment to be given.

5.4 Hyperbaric Oxygen (HBO) Therapy

5.4.1 Treatment delivery

Treatment group

Participants will be compressed to 2.4 ATA (243 kPa) in a hyperbaric chamber and will breathe 100% oxygen. The total time at 2.4 ATA will be 90 minutes. Oxygen is breathed for 30 minutes, followed by a 5-minute "air break" and a further 30 minutes breathing oxygen. A further 5-minute "air break" is followed by a further 30 minutes breathing oxygen. During the final 10 minutes of oxygen breathing, the chamber is depressurised to ambient atmospheric pressure at a linear rate (14.2 kPa/min). Each participant will receive a total of 40* pressure exposures (five days per week for eight weeks).

Control group

Participants will be compressed to 1.3 ATA (131 kPa) in a hyperbaric chamber and will breathe 21% oxygen (air). The total time at 1.3 ATA will be 90 minutes (including 'air breaks' to closely replicate the protocol for the treatment arm). Air is breathed for the duration of the treatment. During the final 10 minutes of the sham exposure, the chamber is depressurised to ambient atmospheric pressure at a linear rate (3 kPa/min). Each participant will receive a total of 40* pressure exposures (five days per week for eight weeks) exactly the same number of exposures as those in the treatment group. 1.3 ATA has been selected for the sham pressure exposure as it is the lowest pressure at which some of the nominated chambers will function.

*The HORTIS trial prescribes 30-40 hyperbaric oxygen therapy sessions. We aim to standardise the treatment for all participants in this study, and have decided on 40 pressure exposures following a review of our own local practices and in consultation with HORTIS.

5.4.2 Monitoring of research subjects during treatment

A physician will be available at the hyperbaric facility during all pressure exposures. There will be a qualified attendant directly observing the subject throughout every session to monitor physical and psychological wellbeing.

Participants will be checked for intercurrent illness before and after hyperbaric treatment by the attending physician.

Adverse events occurring inside the chamber will immediately be notified by the attendant to the physician who will decide on the necessary action (e.g. administration of decongestants, withdrawal of the patient from the chamber). Any patient can be withdrawn from the chamber at any time.

Patients who develop intercurrent upper respiratory tract infection during the course of treatment, and who are unable to equalise their middle ear pressures, will be given standard decongestant therapy. If, despite decongestant therapy, eustachian dysfunction prevents treatment, patients will be given the choice of withdrawal from treatment or having grommets (pressure equalising tubes) inserted into their ears.

Where three or fewer scheduled HBO sessions are missed during the course of treatment, additional sessions will be performed at the end of the 8 weeks trial period to make up a total of 40 treatments. Reasons for withdrawals and any adverse events will be documented and considered in the analysis.

Patients will be asked to produce a list of current medication (including dosage) before they enter into the study and to keep a diary during their HBO therapy. Changes in medication during the 12 months of follow up will be collected at the assessments within 14 days of completing the treatment and at 12 months post randomisation.

5.5 Post-treatment assessments

i) Patient self-assessments within 2 weeks of completion of HBO using IBDQ only (Appendix 1) & at 3, 6, 9 and 12 months after start of HBO therapy or sham treatment using IBDQ and EORTC Quality of Life Questionnaires (QLQ-C30 & QLQ-CR38) (Appendix 4).
ii) Physician/Clinical Nurse Practitioner assessments of late radiation-induced side effects using LENT SOMA scales within 2 weeks of completion of HBO therapy or sham treatment and again at 12 months after start of HBO therapy (Appendix 2).
iii) Physician/Clinical Nurse Practitioner grading of late radiation-induced side effects using the modified CTCAE form, within 2 weeks of completion of HBO therapy or sham treatment and again at 12 months after start of HBO therapy (Appendix 3).
iv) Health economic questionnaire at 3, 6, 9 and 12 months after start of HBO therapy or sham treatment (Appendix 5).
v) Flexible sigmoidoscopy with rectal biopsies and medical photographs of rectal mucosa will be collected within 2 weeks of completion of HBO therapy or sham treatment and again at 12 months after start of HBO therapy (for patients attending The Royal Marsden only).

5.6 Endpoints

5.6.1 Primary clinical endpoints

i) Overall gastrointestinal symptoms score using the IBDQ Quality of life questionnaire (Appendix 1).
ii) Change in rectal IBDQ bleeding score between the two groups (Appendix 1).

5.6.2 Secondary clinical endpoints

i) Physician assessment of rectal dysfunction using LENT SOMA scales of radiation injury (Appendix 2).
ii) Patient self-assessments including urinary and sexual symptoms, using EORTC Quality of Life Questionnaires QLQ-C30 and QLQ-CR38 colorectal cancer module (Appendix 4).
iii) Physician assessment of rectal dysfunction based on the modified CTCAE grading (Appendix 3).

iv) Photographic evidence of changes in images of rectal mucosa taken via flexible sigmoidoscopy.

5.6.3 Translational endpoints

i) Rectal biopsies

Increase in blood vessel density will be investigated using immunohistochemistry on tissue sections. Changes will also be investigated in proteins involved in extracellular matrix metabolism, including fibrogenic cytokines (including CTGF, TGFβ_1), collagen synthesis (including PINP, PIIINP, prolyl-4-hydroxylase) and metalloproteinases (including MMP-I). Changes in mRNA expression will also be investigated (Appendix 6).

5.7 Safety Reporting / Pharmacovigilance

5.7.1 Definitions

Definition of an Adverse Event

An adverse event is any untoward medical occurrence in a patient administered a research procedure; events do not necessarily have a causal relationship with the procedure. For the purpose of this trial, any detrimental change in the patient's condition subsequent to the start of the trial and during the follow-up period, which is not unequivocally due to progression of disease, should be considered as an AE. Whenever one or more signs and/or symptoms correspond to a disease or well-defined syndrome only the main disease/syndrome should be reported. For each sign/symptom the highest grade observed since the last visit should be reported.

Definition of Serious Adverse Events

A Serious Adverse Event (SAE) is defined as an untoward occurrence that:

- results in death;
- is life-threatening;
- requires hospitalisation or prolongation of existing hospitalisation;
- results in persistent or significant disability or incapacity;
- consists of a congenital anomaly or birth defect in a subject's offspring born after the experimental exposure; or
- is otherwise considered medically significant by the investigator.

Definition of Related Adverse Event

A related adverse event is one for which the Chief Investigator or Principal Investigator assesses it resulted from administration of any of the research procedures.

Definition of Unexpected Adverse Event

An unexpected adverse event is any type of event not listed in the protocol as an expected occurrence.

5.7.2 Reporting Procedures

Reporting of Adverse Events

Adverse events will be collected from the time of randomisation to the end of the follow-up period. Adverse events should be recorded in the appropriate section of the CRF.

Reporting of Serious Adverse Events

The PI for each participating site is responsible for reporting SAE's to the CI within 24 hours of the event. The details must be sent by FAX to the Trial Co-ordinator on 020 8661 3107. The SAE form must be completed, signed and dated by the investigator or nominated representative. The PI is responsible for assessing seriousness, causality and expectedness. The Trial Co-ordinator will send the SAE to the Chief Investigator (or nominated representative) for review. The CI is responsible for reporting SAE's to the MHRA, Northern and Yorkshire Research Ethics Committee and the Royal Marsden NHS Foundation Trust in compliance with the current Trust standard operating procedure for reporting SAE's.

Serious Adverse Event follow up
The subject must be followed-up until clinical recovery is complete, laboratory tests have returned to normal, or until disease has stabilised. Information on final diagnosis and outcome of SAE which may not be available at the time the SAE is initially reported should be forwarded to the Trial Co-ordinator.

5.7.3 Expected Adverse Events

Eye refractive change / myopia is a possible related and expected occurrence during the treatment. This is a temporary change and can usually be expected to return to normal within six to twelve weeks of finishing treatment. There is anecdotal evidence that a minority of people experience changes lasting many months, but there is no suggestion in the published literature to indicate that these changes can be permanent.

New cataracts have been observed in patients receiving in excess of 150 hyperbaric oxygen treatments. There is a single case report of a patient who developed a cataract after fewer treatments. This effect can, therefore, be expected but has a very low likelihood of occurring in this study. There is also concern that hyperbaric oxygen therapy can accelerate maturation of existing cataracts although this has not been confirmed in systematic studies. Nevertheless the possibility should be anticipated and existing cataracts should be monitored carefully throughout the study.

Increased fatigue or tiredness in the afternoon following a morning hyperbaric oxygen treatment can be a common side effect for people having repeated treatments.

Some patients receiving 20 treatments or more experience peripheral numbness and tingling, most frequently in the hands. This will resolve within 4 to 6 weeks of stopping the hyperbaric oxygen therapy.

Changes in pressure can cause damage to the middle ear or sinuses if the pressures in them are not equalised. This may cause pain and/ or a ruptured ear drum, which generally heals within a week.

Vigorous efforts to equalise middle ear pressure during pressurisation can cause an inner ear perilymph fistula. Although this is possible, the controlled pressurisation and close monitoring of the patient will prevent provocative pressure exposures and make this outcome very unlikely.

It is also possible for fluid to accumulate in the middle ear over the course of high-pressure treatments, which usually resolves without any specific treatment.

Tooth pain or damage can occur during compression or decompression when gas is trapped within a tooth, under a filling for instance. If there is concern about a patient's teeth, or if they develop dental symptoms, they will be referred to a dental practitioner.

Intercurrent upper respiratory tract infection is also a possible expected but unrelated occurrence during the treatment.

Some patients find the pressure chamber claustrophobic. This is seldom severe enough to prevent them from tolerating the treatment. Short acting anxiolytics can be used but are not generally required. The problem typically improves as the patient becomes more familiar with the environment.

The PI for each site is responsible for logging any expected adverse event on the relevant CRF.

Expected Serious Adverse Events

The list of events below are <u>not subject</u> to expedited notification to R&D, ethics and MHRA as per trust SOP procedures but will be recorded in a CRF / report to the CI.

A very rare side effect is having a fit (seizure). This is short-lived and dealt with by having an air break. There are no lasting effects.

If there are abnormalities in the lung, or the person holds their breath during depressurisation, it is possible for the lungs to rupture and for gas bubbles to enter the blood and travel to the brain causing problems similar to a stroke, or to the heart causing problems similar to a heart attack. The treatment is immediate recompression.

Alternatively the expanding gas can burst through the lining of the lung into the chest cavity causing the lung to collapse. The treatment is removal of the air through a drainage tube placed through the chest wall. Sometimes the gas can cause a pneumomediastinum or subcutaneous emphysema for which there is usually no more treatment than rest and observation although supplemental oxygen can be helpful.

When people are exposed to increased pressure and that pressure is reduced too rapidly, it is possible for excess dissolved gas (usually nitrogen) to form bubbles in blood and body tissues causing decompression illness. The most common symptoms are pain in or around a joint and nervous system problems.

These complications are extremely unlikely in the controlled environment of a high pressure chamber where the pressure is reduced slowly.

5.7.4 Urgent Safety Measures

There will be a qualified attendant directly observing the subject throughout every session to monitor physical and psychological wellbeing. A physician will be available at the hyperbaric facility during all pressure exposures who will decide on the necessary course of action. See sections 5.7.2.

5.7.5 DSURs and Annual Progress Report

The CI is responsible for producing an annual DSUR in liaison with the Pharmacovigilance Officer in the Trust Clinical R&D office, which will be sent to the MHRA. An Annual Progress Report will be submitted to the Main Research Ethics Committee.

6 STATISTICAL CONSIDERATIONS

6.1 Stratification

Patients will be stratified according to the severity of their symptoms.

6.2 Principal Endpoints

6.2.1 Primary endpoints

i) Gastrointestinal symptoms score using the IBDQ Quality of life questionnaire will be used as the primary endpoint. This scoring system may be more sensitive to small differences than LENT SOMA and is also an assessment of change in patient QoL. The analysis of the primary endpoint will be the change in score from baseline to 12 months after start of HBO therapy.
ii) The second primary endpoint will be to compare the change (12 month minus baseline) in rectal IBDQ bleeding score between the two groups.

6.2.2 Secondary endpoints

i) Proportion of items graded as marked or severe (grade 3 or 4) (as below).
ii) Physician assessments of adverse effects using LENT SOMA scales.
iii) Physician assessment of rectal dysfunction using the modified CTCAE grading.
iv) Patient self-assessments: QLQ–C30 and 38.
v) Photographic assessments of rectal mucosa.
vi) Evaluation of health economics data.

6.2.3 Translational endpoints (see Appendix 6)

i) Rectal biopsies: Changes in expression of mRNA and proteins involved in extracellular matrix metabolism from baseline (pre-treatment) to the end of HBO therapy, including fibrogenic cytokines (incl. CTGF, $TGF\beta_1$), collagen synthesis (incl. PINP, PIIINP, prolyl-4-hydroxylase) and metalloproteinases (incl. MMPI). Increase in blood vessel density will also be investigated.

6.3 Sample Size

The sample size has been based on changes in the Modified Inflammatory Bowel Disease Questionnaire in patients who improved, remained stable or deteriorated after one month on therapy for chronic inflammatory bowel disease (Crohn's disease**)** [49]. These changes were 15 ±10 (n=109), 3 ±7 (n=63), and -3 ±6 (n=8), respectively. We therefore used 10 as an estimate of the standard deviation of the change at 12 months, assuming it will be slightly higher than the difference seen at 1 month. The IBDQ endpoint will be a minimum clinically worthwhile difference of 7.5 (this was increased from 7 by a substantial amendment in February 2012). This implies we need 75 patients (50 HBO:25 placebo) for 80% power employing a two-sided 3% significance level). It is estimated that 40% of patients will have grade 2, 3 or 4 bleeding, expected to comprise 30 of the 75 patients. The reduction in bleeding in this group of patients will be compared between randomised groups. A large reduction in the proportion of patients showing a fall in their grade of rectal bleeding at 12 months will be

detectable, e.g. from 80% showing a fall in the HBO group to 10% in the placebo group (80% power, 2% two sided significance level). The overall false positive rate is therefore 5%. The pattern of rectal bleeding change in the patients with grade 0-1 bleeding at baseline will be reported but not formally compared between arms, this group is of less interest because bleeding does not represent a major impediment to their daily living and they are very unlikely to develop serious rectal bleeding after randomisation into HOT II.

6.4 Analysis

Primary endpoints

The Modified Inflammatory Bowel Disease score is the first primary endpoint, the second primary endpoint is the IBDQ rectal bleeding score. Analysis will be carried out on an intention to treat basis. The mean difference in change from baseline to 12 months in both trial arms will be compared using the Mann-Whitney U test, or unpaired t-test if the values are approximately normally distributed. If necessary, multivariate analysis will be used to adjust for any potential confounding factors, though the ability to use multivariate analysis will be limited by the fact there are only 25 patients in one arm. The impact of missing values will be investigated by using techniques such as LOCF.

Secondary Endpoints

i-ii) Changes in the Subjective descriptor LENT SOMA will be a secondary endpoint. Within each of three LENT SOMA descriptors (Subjective, Objective, Management), individual parameters are assessed on a 4-point scale. The EORTC and RTOG suggest that the descriptors can be used to develop a score for each normal tissue, by summing numerical scores of individual parameters [95]. The aggregate Subjective parameter score will be used to reflect deterioration or improvement in the severity of late normal tissue effects, by an increase or decrease, respectively, in its aggregate score.

There will be no formal statistical analysis of the other secondary endpoints but the descriptive nature of these results will be used to strengthen the interpretation of changes in the primary endpoint. Several analyses will be carried out including but not limited to the following:

iii) Changes in modified CTCAE grades.
iv) Standard procedures will be followed for describing quantitative changes in QoL domains using EORTC Quality of Life Questionnaires (QLQ-C30 and CR38).
v) Photographs of rectal mucosa will be scored by two experienced endoscopists, who will be blinded with respect to treatment allocation and time of assessment (pre- or post-treatment). The mucosal appearance will be scored using the Wachter classification [135]. Disagreement in any scores will be resolved by arbitration after discussion with a third independent endoscopist. Differences in the appearance before and after treatment between the two groups will be assessed.
vi) Evaluation of health economics data.

6.5 Frequency of analyses

There will be a sequential monitoring of toxicity, and the first analysis will be carried out when the first 21 patients have completed 12 months of assessment. Toxicity and the frequency and nature of adverse events will be compared between the randomised groups. Summary measures and non-parametric tests will be used as necessary. In particular, the proportion of patients experiencing toxicity grade 3 or 4 and the maximum toxicity grade will be compared. An analysis of safety endpoints by treatment received will be performed.

7 RESEARCH GOVERNANCE

7.1 Trial Administration and Logistics

The Royal Marsden Foundation Trust and The Institute of Cancer Research are joint sponsors of this study in line with the Research Governance Framework for Health and Social Care and the principles of Good Clinical Practice (GCP).

7.1.1 Chief Investigator

The Chief Investigator is Professor John Yarnold who will ensure that the trial is conducted in accordance with Good Clinical Practice. Professor Yarnold has final responsibility for all trial authorisations (including submission to RM Committee for Clinical Research, NRES and MHRA) and reporting requirements.

7.1.2 Participating centres responsibilities

Responsibilities are defined in an agreement between an individual participating centre and the Sponsors.

All participating hyperbaric medicine units operate under the terms of the Healthcare Commission (HCC) terms. In order to comply with HCC regulations to operate a clinical hyperbaric oxygen treatment facility all units will follow "A European Code of Good Practice for Hyperbaric Oxygen Therapy (2004)*, in conjunction with:

- A Planned maintenance programme within the guidelines of the chamber manufacturer or IMCA (International Marine Contractors Association) standards.
- An Authorised person for HTM 0022-02 (Health Technical Memoranda), MGPA (Medical Gas Pipework System) approved.
- Minimum air quality – BSEN 12021 standard
- Minimum oxygen quality – Medical Grade

* http://www.oxynet.org/02COSTinfo/Public/ECGP%20for%20HBO%20-%20May%202004.pdf

7.1.3 IMP Management

Gary Smerdon, (Research Director for The Diving Diseases Research Centre, Plymouth) will act as a reference point in the event of any advice required regarding the IMP or its use during the trial.

7.2 Case Report Forms (CRFs)

A series of questionnaires will be completed by both the patient and research team at set time points during the study in order to assess the patient's late radiotherapy side effects and quality of life. The CRFs completed at the sites should be signed and dated and forwarded to the Trial Coordinator once completed. The CRFs will not be made available to people outside of the research team, however, access will be granted for audit and monitoring purposes and will be provided to regulatory authorities, Ethics Committee or other relevant ICR or Trust personnel.

Document	Appen-dix	Completion by
Modified IBDQ Quality of Life Questionnaire	1	Patient
LENT SOMA late radiotherapy toxicity scoring form	2	RMH Physician / Clinical Nurse Practitioner
Modified CTCAE Grading Form	3	Local Gastroenterologist (pre-trial entry) / RMH Physician / Clinical Nurse Practitioner (post-trial entry)
EORTC QLQ-C30 and QLQ-CR38 self assessment forms	4	Patient
Health Economics Questionnaire	5	Patient
Eligibility assessment forms and CRF's not included in trial protocol:		
Pre-Trial Gastroenterology Form	n/a	Patient and referring gastroenterologist
Eligibility Checklist	n/a	RMH Physician
Pre-Randomisation and Pre-Treatment Checklist	n/a	RMH Physician / Trial Co-ordinator
Post Treatment Assessment Form	n/a	RMH Physician / Trial Co-ordinator
Treatment Compliance Form	n/a	Hyperbaric Unit
Treatment Deviation Form	n/a	Hyperbaric Unit
Adverse Event Log	n/a	Trial Co-ordinator

7.3 Protocol compliance

This trial is being conducted in accordance with the professional and regulatory standards required for non-commercial research in the NHS under the Research Governance Framework for Health and Social Care and GCP.

By participating in the HOT II Trial the Principal Investigators at each centre are confirming agreement with his/her local NHS Trust to ensure that:

- sufficient data is recorded for all participating patients to enable accurate linkage between hospital records and CRF's
- source data and all trial related documentation are accurate, complete, maintained and accessible for monitoring and audit visits
- all original Consent Forms are dated and signed by both the patient and investigator, and are kept together in a central log together with a copy of the specific patient information sheet they were given at the time of consent
- copies of CRF's are retained for 15 years to comply with international regulations
- staff will comply with the Standard Operating Procedures for HOT II.

The Trial Co-ordinator will monitor receipt of CRF's and will also check incoming CRF's for compliance with the protocol, inconsistent and missing data.

7.4 Treatment compliance/deviation

It is the responsibility of each hyperbaric medicine unit to complete a "Treatment Compliance Form for each volunteer who is randomised to take part in the trial.

If the volunteer does not receive their hyperbaric treatment as per the trial protocol, the unit must also complete a "Deviation Form"

If a volunteer's condition deteriorates during HBO therapy, and their physician requires them to be hospitalised for an infusion or further investigations and treatment, they can be temporarily removed from the trial. HBO therapy in accordance with their original randomisation will be resumed on completion of the referral, unless the treating hyperbaric physician decides against this in liaison with the CI. A copy of the "Deviation Form" as well as "Treatment Compliance Form" must be completed for such volunteers.

All completed forms should be signed and sent via hard copy or email to the CI, Professor John Yarnold, The Royal Marsden, Downs Road, Sutton, Surrey, SM2 5PT /john.yarnold@icr.ac.uk

7.5 Treatment withdrawal

Patients may be withdrawn from the study for the following reasons:

- Disease progression before completion of study treatment.
- Withdrawal of consent for treatment and/or study participation.
- Unacceptable treatment related toxicity or adverse events as judged by either the subject's treating physician or the Chief Investigator.
- Pregnancy.
- Patient non-compliance.
- Death.

7.6 Direct Access to Source Data

On a day to day basis only members of the research team will have direct access to data and documents pertaining to this study. Access for audit purposes will be provided to regulatory authorities, Ethics Committee or other relevant ICR or Trust personnel.

The Case Report Form will be considered source data for this study as the forms are questionnaires completed directly by patients and/or clinical staff. Thus the information will not be recorded in the medical notes or on the Hospital Information System. However, consent and eligibility should be documented in the medical notes and this will be checked at random as part of the monitoring for this study.

Key eligibility components to be recorded on the Royal Marsden Hosptial Electronic Patient Record System (please see section 4).

7.7 Trial Management

7.7.1 Trial Management Group

A Trial Management Group (TMG) will be set up and will include the Chief Investigator, Co-investigators and identified Collaborators, the Trial Statistician and the Trial Co-ordinator. Principal investigators and key study personnel will be invited to join the TMG as appropriate to ensure representation from a range of centres and professional groups.

Notwithstanding the legal obligations of the Sponsor and Chief Investigator, the TMG have operational responsibility for the conduct of the trial.

7.7.2 Trial Steering Committee

A Trial Steering Committee (TSC) will monitor and supervise the progress of the trial. The role of the TSC is to provide overall supervision of the trial on behalf of the funding body. In particular, the TSC will concentrate on the progress of the trial, adherence to the protocol, patient safety and the consideration of new information. Day-to-day management of the trial is the responsibility of the Chief Investigator and TMG.

Membership will be limited and include an independent Chair (not involved directly in the trial other than as a member of the TSC), not less than two other independent members, the Chief Investigator and the Trial Statistician.

The Trial co-ordinator and other key members of the TMG will attend meetings (as observers) as appropriate. Observers from the funding body and, if applicable, host Institutions or sponsors will be invited to all meetings. The TSC will meet at least annually.

7.7.3 Data Monitoring and Ethics Committee

An independent Data Monitoring and Ethics Committee (DMEC) will be established to oversee the safety and interim efficacy of the trial. This committee will be constituted according to MRC Good Clinical Practice (MRC GCP). The DMEC will meet on a regular basis as they see fit, but no less than annually. Following each meeting, the DMEC will report their findings and recommendations to the TSC and to the TMG.

Interim analysis (split by treatment group) of IBDQ, Subjective LENT-SOMA score, modified CTCAE grading, EORTC QLQ-C30 and 38, side-effects, tolerability and other endpoints for all randomised patients will be supplied in strict confidence by the trial statisticians to the DMEC together with any other analyses that the DMEC may request. The complete DMEC reports will remain confidential to the DMEC members and statisticians providing the report, however the Chief Investigator and Trial Co-ordinator will receive subsets of the report as seen fit by the DMEC (e.g. accrual, compliance and data completeness). Basic accrual data and safety reports, aggregated across the two treatment groups will be produced at appropriate periodic intervals and distributed to the TMG.

The main criterion for early stopping of the trial by the TSC upon suggestion from the DMEC will be that evidence from the trial or from other sources supplies a) proof beyond reasonable doubt that for all, or for some types of patient, one treatment regimen is clearly indicated or contra-indicated in terms of a net difference in therapeutic effect or b) evidence that might reasonably be expected to influence routine clinical practice. Criteria for the above will usually be a difference in IBDQ significant at $p<0.001$.

No results will be made available to participants or the sponsor until the DMEC consider the results to be clinically and statistically informative. The DMEC may recommend continuation beyond the planned number of patients in the trial if it is felt that further information is required to address reliably the hypothesis in question.

7.8 End of study

The study end date is deemed to be the date of the last data capture.

7.9 Archiving

Essential documents are documents that individually and collectively permit evaluation of the conduct of the trial and the quality of the data produced, for example CRFs, patient consent forms. These will be maintained in Prof Yarnold's Research Office at The Royal Marsden and at the Investigator Sites in a way that will facilitate the management of the trial, audit and inspection. They will be retained for a sufficient period (at least 15 years) for possible audit and inspection by the regulatory authority. The sponsor or trial organisers will notify the investigator sites of their responsibility for archiving essential documents. Documents will be securely stored and access will be restricted to authorised personnel. An archive log will be maintained to track archived documents.

7.10 Publishing policy

All publications and presentations relating to the trial will be authorised by the TMG. Authorship will be determined by the TMG and will include the Chief Investigator, Co-investigators, Collaborators, and Trial Statistician.

Authors will only present data separately to the total data available, with the permission of the TMG, and not less than 6 months after publication of the main results.

8 CONFIDENTIALITY AND LIABILITY

8.1. Risk assessment

Generic Risk Assessment hazards to patients, study and organisation have been performed for the trial.

8.2. Liability/Indemnity/Insurance

This study is an investigator-led trial (endorsed by the Clinical Trials Awards and Advisory Committee (CTAAC) of Cancer Research UK. Indemnity for participating NHS hospitals is provided by the usual NHS indemnity arrangements. The participating hyperbaric treatment units have their own indemnity arrangements in place to cover incidents of both clinical and general nature.

8.3 Patient Confidentiality

The patient's full name, date of birth, hospital number and NHS number will be collected at randomisation to allow tracing through national records. The personal data recorded on all documents will be regarded as confidential, and to preserve each subject's anonymity, only their Trial ID and screening number will be recorded on subsequent Case Report Forms. Patient addresses will be requested for distribution of quality of life questionnaires.

The investigators and hyperbaric treatment units must keep a separate log of patients' trial numbers, names, and hospital numbers. They must maintain in strict confidence trial documents, which are to be held in the local hospital or hyperbaric treatment unit (e.g. patients' written consent forms). The investigators and hyperbaric treatment units must ensure the patient's confidentiality is maintained.

Prof Yarnold's Research Office will maintain the confidentiality of all subject data and will not reproduce or disclose any information by which subjects could be identified, other than reporting of serious adverse events. Representatives of the trial team will be required to have access to patient notes for quality assurance purposes but patients should be reassured that their confidentiality will be respected at all times. In the case of special problems and/or competent authority queries, it is also necessary to have access to the complete study records, provided that patient confidentiality is protected.

8.4 Ethical Considerations

It is the responsibility of the Chief Investigator to obtain a favourable ethical opinion (main REC approval) prior to recruitment of patients in to the study.

It is the responsibility of the Principal Investigator at each participating Trust to obtain site-specific approval of the trial protocol and any subsequent amendments. All correspondence with the local REC should be filed by the local Investigator.

It is the responsibility of the Chief Investigator or nominated representative to give each patient, prior to inclusion in the trial, full and adequate verbal and written information regarding the objective and procedures of the trial and the possible risks involved. Patients must be informed about their right to withdraw from the trial at any point. Written patient information must be given to each patient before enrolment. The written patient information is an approved patient information sheet according to national guidelines.

It is the responsibility of the Chief Investigator to obtain signed informed consent from all patients prior to inclusion in the trial, as per the RMH/ ICR SOP for Obtaining Informed Consent in Research gSOP-04 as amended.

8.5 Sample storage

All retained tissue samples will be processed, stored and used in accordance with the Human Tissue Act 2004.

9 FINANCIAL MATTERS

The trial is investigator designed and led, and has been approved by the Clinical Trials Awards and Advisory Committee (CTAAC). It is endorsed by Cancer Research UK and meets the criteria for R&D support as outlined in the Statement of Partnership on Non-Commercial R&D in the NHS in England.

Research costs are being funded by Cancer Research UK, excess treatment costs by the Department of Health. If additional financial support is received from any other source, this will be made apparent to the approving main REC and CTAAC, but will not require a protocol amendment.

No individual per patient payment will be made to trusts or investigators, but NCRN (or regional equivalent) network resources should be made available as the trial is part of the NCRI portfolio by virtue of its approval by CTAAC.

10 APPENDICES

Appendix 1: Modified IBDQ

Appendix 2: LENT SOMA late radiotherapy toxicity scoring form

Appendix 3: Modified CTCAE Grading Form

Appendix 4: EORTC QLQ-C30 and EORTC QLQ-CR38 patient self-assessment forms

Appendix 5: Health Economic Questionnaire

Appendix 6: Mechanisms of tissue response to hyperbaric oxygen after radiotherapy

Appendix 1 **Modified IBDQ** HOT II Vs 4 Dated 24.03.2011 (dec13 version ibdq)

Trial Number:	**Screening Number:**	**Baseline / 2wks / 3 mths / 6mths / 9 mths / 12 mths**
Date:		

In the last two weeks please tell us how often you have:

		More than ever before	Extremely frequently	Very frequently	Moderate increase in frequency	Some increase in frequency	Slight increase in frequency	Not at all / normal
1	had your bowel open?							
2	felt tired and worn out?							
3	felt frustrated, impatient or restless?							
4	been unable to do what you want because of your bowels?							
5	had loose bowel movements?							
6	worried about your energy levels?							
7	worried about having to have something done about your bowels?							
8	you had to cancel an engagement because of your bowels?							
9	been troubled by pain in your bottom?							
10	felt generally unwell?							
11	worried about not being able to find a lavatory?							
12	been prevented doing leisure or sports by your bowels?							
13	been troubled by cramp in your tummy or bottom?							
14	been waking at night or having difficulty sleeping?							

		More than ever before	Extremely frequently	Very frequently	Moderate increase in frequency	Some increase in frequency	Slight increase in frequency	Not at all / normal
15	been depressed or discouraged?							
16	not gone somewhere because there is no lavatory nearby?							
17	passed a large amount of gas							
18	worried about getting to the weight you would like							
19	worried about your illness							
20	been troubled by bloating							
21	been relaxed and free from tension							
22	had a problem with bleeding from your bottom?							
23	been embarrassed about your bowels?							
24	felt like you need to have your bowels open but nothing happens?							
25	felt tearful and upset?							
26	been troubled by accidental soiling?							
27	felt angry as a result of your bowel problems?							
28	felt limited in sexual activity because of your bowels?							
29	felt disgusted about your bowel problems?							
30	felt irritable?							
31	experienced a lack of understanding from others?							
32	felt satisfied, happy or pleased with your life?							

Trial Number:	**Screening Number:**

SOMA Late Radiotherapy Toxicity - RECTUM

Assessment	Baseline	2 wks post treatment	12 months
Date			

Grade 1	Grade 2	Grade 3	Grade 4

Subjective

Stool Frequency				2-4 per day	5-8 per day	>8 per day	Uncontrolled Diarrhoea
Sphincter Control				Occasional	Intermittent	Persistent	Refractory
Pain				Occasional & minimal	Intermittent & tolerable	Persistent & intense	Refractory & excruciating
Tenesmus				Occasional urgency	Intermittent urgency	Persistent urgency	Refractory
Mucosal Loss				Occasional	Intermittent	Persistent	Refractory

Objective

Bleeding				Occult	Intermittent >2/week	Persistent, daily	Gross haemorrage
Stricture				>2/3 normal diameter with dilation	1/3 -2/3 normal diameter with dilation	< 1/3 normal diameter	Complete obstruction
Ulceration				Superficial ≤ = 1cm2	Superficial > 1cm2	Deep Ulcer	Perforation, fistula

Management

Pain				Occasional Non Narcotic	Regular Non Narcotic	Regular Narcotic	Surgical
Tenis/ frequency				Occasional , ≤ 2 antidiarrhoeals/wk	Regular, >2 anti diarrhoeals/wk	Multiple,≤ 2 antidiarrhoeals/day	Surgical intervention/ permanent colostomy
Bleeding				Stool Softener ,iron therapy	Occasional transfusion	Frequent Transfusions	Surgical intervention/ permanent colostomy
Stricture				Diet modification	Occasional dilation	Regular Dilation	Surgical intervention/ permanent colostomy
Ulceration				Diet modification, stool softener	Occasional steroids	Steroids per enema / Hyperbaric Oxygen	Surgical intervention/ permanent colostomy
Sphincter control				Occasional use of incontinence pads	Intermittent use of incontinence pads	Persistent use of incontinence pads	Surgical intervention/ permanent colostomy

Analytic: Not required

Trial Number:	**Screening Number:**

SOMA Late Radiotherapy Toxicity - SMALL INTESTINE/COLON

Assessment	Baseline	2 wks post treatment	12 months
Date			

Grade 1	Grade 2	Grade 3	Grade 4

Subjective

Stool Frequency				2-4 per day	5-8 per day	>8 per day	Refractory Diarrhoea
Stool Consistency				Bulky	Loose	Mucous, dark, watery	
Pain				Occasional – minimal	Intermittent - tolerable	Persistent - intense	Refractory – rebound
Constipation				3-4 week	Only 2 per week	Only 1 per week	No stool in 10 days

Objective

Melena				Occult/Occasional	Intermittent & tolerable, normal Hb	Persistent ,10-20% decrease Hb	Refractory or frank blood > 20% Hb
Wt loss fromRT				>=5%-10%	>10%-20%	>20-30%	>30%
Stricture				>2/3 normal diameter with dilation	1/3 -2/3 normal diameter with dilation	< 1/3 normal diameter	Complete obstruction
Ulceration				Superficial < = 1cm2	Superficial > 1cm2	Deep Ulcer	Perforation, fistula

Management

Pain				Occasional non - narcotic	Regular non- narcotic	Regular narcotic	Surgical Intervention
Stool consist/ freq				Diet Modification	Reg. Use non-narcotic anti diarrhoeal	Continuous Use narcotic anti diarrhoeal	
Bleeding				Iron Therapy	Occasional Transfusion	Frequent Transfusions	Surgical Intervention
Stricture				Occasional diet adaptation	Diet adaptation required	Medical intervention, NG suction	Surgical Intervention
Ulceration						Medical Intervention	Surgical Intervention

Analytic: Not required

Completed by: Name _____ **Signature** _____ **Date** _____

Trial Number _____ Screening Number _____ Date _____

Please record the grade of disorders in the right-hand column: if no symptoms, leave the box blank. Baseline / 2wks post / 12 months

	Gastrointestinal Disorders - Grade					
	Adverse Event	**1**	**2**	**3**	**4**	**Grade**
1	**Abdominal Pain**	Mild Pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	-	
	Definition: A disorder characterized by a sensation of marked discomfort in the abdominal region.					
2	**Bloating**	No change in bowel function or oral intake	Symptomatic, decreased oral intake; change in bowel function	-	-	
	Definition: A disorder characterized by subject-reported feeling of uncomfortable fullness of the abdomen.					
3	**Constipation**	Occasional or intermittent symptoms; occasional use of stool softeners, laxatives, dietary modification, or enema	Persistent symptoms with regular use of laxatives or enemas; limiting instrumental ADL	Obstipation with manual evacuation indicated; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	
	Definition: A disorder characterized by irregular and infrequent or difficult evacuation of the bowels.					
4	**Diarrhoea**	Increase of <4 stools per day over baseline; mild increase in ostomy output compared to baseline	Increase of 4 - 6 stools per day over baseline; moderate increase in ostomy output compared to baseline	Increase of >=7 stools per day over baseline; incontinence; hospitalization indicated; severe increase in ostomy output compared to baseline; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	
	Definition: A disorder characterised by frequent and watery bowel movements.					
5	**Faecal Incontinence active = urge incontinence passive = leakage**	Occasional use of pads required	Daily use of pads required	Severe symptoms; elective operative intervention indicated	-	
	Definition: A disorder characterised by the inability to control the escape of stool from the rectum.					
6	**Flatulence**	Mild symptoms; intervention not indicated	Moderate; persistent; psychosocial sequelae	-	-	
	Definition: A disorder characterized by a state of excessive gas in the alimentary canal.					

	Gastrointestinal Disorders - Grade					
	Adverse Event	**1**	**2**	**3**	**4**	**Grade**
7	**Frequency**	Mild, intervention not indicated	Moderate symptoms, limiting instrumental ADL	Severe symptoms; limiting self care ADL	-	
	Definition: A disorder characterised by the need to pass stool much more frequently than is normal.					
8	**Rectal bleeding**	Mild; intervention not indicated	Moderate symptoms; medical intervention or minor cauterization indicated.	Transfusion, radiologic, endoscopic, or elective operative intervention indicated	Life-threatening consequences; urgent intervention indicated	
	Definition: A disorder characterized by bleeding from the rectal wall and discharged from the anus.					
9	**Rectal pain**	Mild pain	Moderate pain; limiting instrumental ADL	Severe pain; limiting self care ADL	-	
	Definition: A disorder characterised by a sensation of marked discomfort in the rectal region.					
10	**Tenesmus**	Mild, intervention not indicated	Moderate symptoms; limiting instrumental ADL	Severe pain; limiting self care ADL	-	
	Definition: The sensation of feeling the rectum is still not empty even after passing a stool.					
11	**Urgency**	Mild, intervention not indicated	Moderate symptoms; limiting instrumental ADL	Severe pain; limiting self care ADL	-	
	Definition: A disorder characterised by the desperate need to open the bowel.					
12	**Gastrointestinal disorders –** **Other, specify below :**	Asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated	Moderate; minimal, local or non-invasive intervention indicated; limited age-appropriate instrumental ADL	Severe or medically significant but not immediately life-threatening; hospitalisation or prolongation of existing hospitalisation indicated; disabling; limiting self care ADL	Life-threatening consequences; urgent intervention indicated	

ADL – activities of daily living Instrumental ADL refers to preparing meals, shopping etc Self care ADL refers to bathing, dressing, feeding self etc

Completed by: Name _____ **Signature** _____ **Date** _____

EORTC QLQ-C30 (version 3)

We are interested in some things about you and your health. Please answer all of the questions yourself by circling the number that best applies to you. There are no "right" or "wrong" answers. The information that you provide will remain strictly confidential.

Trial Number: _____ / Screening No: _____

Please fill in today's date (Day, Month, Year): _____ / _____ / _____

		Not at All	A Little	Quite a Bit	Very Much
1.	Do you have any trouble doing strenuous activities, like carrying a heavy shopping bag or a suitcase?	1	2	3	4
2.	Do you have any trouble taking a long walk?	1	2	3	4
3.	Do you have any trouble taking a short walk outside of the house?	1	2	3	4
4.	Do you need to stay in bed or a chair during the day?	1	2	3	4
5.	Do you need help with eating, dressing, washing yourself or using the toilet?	1	2	3	4

During the past week:

		Not at All	A Little	Quite a Bit	Very Much
6.	Were you limited in doing either your work or other daily activities?	1	2	3	4
7.	Were you limited in pursuing your hobbies or other leisure time activities?	1	2	3	4
8.	Were you short of breath?	1	2	3	4
9.	Have you had pain?	1	2	3	4
10.	Did you need to rest?	1	2	3	4
11.	Have you had trouble sleeping?	1	2	3	4
12.	Have you felt weak?	1	2	3	4
13.	Have you lacked appetite?	1	2	3	4
14.	Have you felt nauseated?	1	2	3	4
15.	Have you vomited?	1	2	3	4

Please go on to the next page

During the past week:		Not at All	A Little	Quite a Bit	Very Much
16.	Have you been constipated?	1	2	3	4
17.	Have you had diarrhea?	1	2	3	4
18.	Were you tired?	1	2	3	4
19.	Did pain interfere with your daily activities?	1	2	3	4
20.	Have you had difficulty in concentrating on things, like reading a newspaper or watching television?	1	2	3	4
21.	Did you feel tense?	1	2	3	4
22.	Did you worry?	1	2	3	4
23.	Did you feel irritable?	1	2	3	4
24.	Did you feel depressed?	1	2	3	4
25.	Have you had difficulty remembering things?	1	2	3	4
26.	Has your physical condition or medical treatment interfered with your family life?	1	2	3	4
27.	Has your physical condition or medical treatment interfered with your social activities?	1	2	3	4
28.	Has your physical condition or medical treatment caused you financial difficulties?	1	2	3	4

For the following questions please circle the number between 1 and 7 that best applies to you

29. How would you rate your overall health during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

30. How would you rate your overall quality of life during the past week?

1	2	3	4	5	6	7
Very poor						Excellent

EORTC QLQ – CR38

Patients sometimes report that they have the following symptoms or problems. Please indicate the extent to which you have experienced these symptoms or problems during the past week. Please answer by circling the number that best applies to you.

Trial Number: _____ / Screening No: _____

Please fill in today's date (Day, Month, Year): _____ / _____ / _____

During the past week :	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
31. Did you urinate frequently during the day?	1	2	3	4
32. Did you urinate frequently during the night?	1	2	3	4
33. Did you have pain when you urinated?	1	2	3	4
34. Did you have a bloated feeling in your abdomen?	1	2	3	4
35. Did you have abdominal pain?	1	2	3	4
36. Did you have pain in your buttocks?	1	2	3	4
37. Were you bothered by gas (flatulence)?	1	2	3	4
38. Did you belch?	1	2	3	4
39. Have you lost weight?	1	2	3	4
40. Did you have a dry mouth?	1	2	3	4
41. Have you had thin or lifeless hair as a result of your disease or treatment?	1	2	3	4
42. Did food and drink taste different from usual?	1	2	3	4
43. Have you felt physically less attractive as a result of your disease or treatment?	1	2	3	4
44. Have you been feeling less feminine/masculine as a result of your disease or treatment?	1	2	3	4
45. Have you been dissatisfied with your body?	1	2	3	4
46. Were you worried about your health in the future?	1	2	3	4

During the past <u>four</u> weeks:	**Not at All**	**A Little**	**Quite a Bit**	**Very Much**
47. To what extent were you interested in sex?	1	2	3	4
48. To what extent were you sexually active (with or without intercourse)?	1	2	3	4
49. Answer this question only if you have been sexually active: To what extent was sex enjoyable for you?	1	2	3	4

Please go on to the next page

During the past four weeks:

	Not at All	A Little	Quite a Bit	Very Much
For men only:				
50. Did you have difficulty getting or maintaining an erection?	1	2	3	4
51. Did you have problems with ejaculation (e.g., so-called "dry ejaculation")?	1	2	3	4
Only for women who have had intercourse:				
52. Did you have a dry vagina during intercourse?	1	2	3	4
53. Did you have pain during intercourse?	1	2	3	4

54. Do you have a stoma (colostomy bag)? **No** **Please answer questions 55 to 61**

(Please circle No or Yes) **Yes** **Please skip questions 55 to 61 and answer questions 62 to 68**

During the past week:

	Not at All	A Little	Quite a Bit	Very Much
Only for patients WITHOUT a stoma (colostomy bag):				
55. Did you have frequent bowel movements during the day?	1	2	3	4
56. Did you have frequent bowel movements during the night?	1	2	3	4
57. Did you feel the urge to move your bowels without actually producing any stools?	1	2	3	4
58. Have you had any unintentional release of stools?	1	2	3	4
59. Have you had blood with your stools?	1	2	3	4
60. Have you had difficulty in moving your bowels?	1	2	3	4
61. Have your bowel movements been painful?	1	2	3	4
Only for patients WITH a stoma (colostomy bag):				
62. Were you afraid that other people would be able to hear your stoma?	1	2	3	4
63. Were you afraid that other people would be able to smell your stools?	1	2	3	4
64. Were you worried about possible leakage from the stoma bag?	1	2	3	4
65. Did you have problems with caring for your stoma?	1	2	3	4
66. Was your skin around the stoma irritated?	1	2	3	4
67. Did you feel embarassed because of your stoma?	1	2	3	4
68. Did you feel less complete because of your stoma?	1	2	3	4

Appendix 5 **Version 3, 24.03.2011**

Health Economics Questionnaire

Trial Number:	**Screening Number:**	**Baseline / 3 mths / 6mths / 9 mths / 12 mths**
Date:		

We would like to find out about the impact of your urinary and bowel problems in relation to costs to you and also the impact on the health service.

1. Have you seen any health care professionals **during the last 3 months**, because of your urinary or bowel problems.

Type of person consulted	**Yes**	**No**	**If Yes, total number of visits**
Have you seen your GP at a health centre or surgery?			
Have you seen a nurse at a health centre or surgery?			
Have you seen your GP in your own home?			
Have you seen a nurse in your own home?			
Have you seen a care worker in your own home?			
Have you seen another health professional,? Please specify type? e.g dietician…….			
Have you seen another health professional? Please specify type? …….			

2. Have had any <u>inpatient</u> and / or <u>outpatient</u> **days during the last 3 months**, because of your urinary or bowel problems.

Type of hospital visit	**Yes**	**No**	**If Yes, total number of appointments / days**
Have you had any outpatient hospital appointments?			
Have you had any inpatient days?			

3. Please provide details of any surgical or other medical procedures which you have undergone **during the last 3 months.**

Type of procedure	**Yes**	**No**	**If Yes, please give the date**
CT / MRI Scan			
SeHCAT Scan			
Upper GI Endoscopy			
Colonoscopy / flexible sigmoidoscopy			
Other: (Please provide details)			

4. **During the past 3 months**, has your GP or Consultant prescribed any medications or dietary supplements?

(please circle) **Yes** **No**

If Yes, please give the following information for each of the medications / supplements prescribed:

Name of medication / supplement	**How long did you use/ have you been using the medication/ supplement?**
	weeks
	weeks
	weeks
	weeks

5. **During the past 3 months,** has the health service or social services provided you with any incontinence aids eg pants, pads, sheets for your bowel or urinary problems? (please circle) **Yes** **No**

If Yes, please give the following information for each:

Type of incontinence aid	Number provided over the last 3 months	Were you CHARGED for these devices? Yes	No	N/A
Pads				
Pants				
Sheets				
Other				

6. **During the last 3 months** which of the following best describes yourself (or your day to day activities)? You may tick more than one box.

Activity	Tick if applicable
Full-time employment	
Part time employment	
Self-employed	
Voluntary work	
Household work	
Seeking work	
Part time student	
Full time student	
Retired	
Sick Leave	
Other	

<u>In relation to any paid activities</u> (if applicable):

7. During the days you do **paid work**, please indicate on the scale of 1 to 10, how much your bowel or urinary problems impact on your ability to do your work.

0 1 2 3 4 5 6 7 8 9 10

Unable to perform tasks

Perform tasks as well as before diagnosis

8. **During the last 3 months** how many hours / days have you taken off work, because of your urinary or bowel problems, including time spent travelling to and visiting your family doctor or other health professionals.

_____ **hours / days***

(*delete as appropriate)

9. Please tick any of the following that best describes the arrangements your employer made or you made to cover the time you were off work **during the last 3 months.**

Type of Cover	**Tick if applicable**
Employed temporary workers	
Hired new worker	
Made up by you on return to work (during normal working hrs)	
Made up by you during extra working hrs	
Covered by other existing employees (during normal working hours)	
Covered by other existing employees (during extra working hours)	
Nothing	
Don’t know	
Other: please specify	

<u>In relation to unpaid activities</u> (if applicable):

10. During the last 3 months have your urinary or bowel problems prevented you carrying out any of the following activities: shopping, odd jobs, chores, work in the household, caring for others eg children, parents, voluntary work?

(please circle) **Yes** **No**

If Yes:
11. How much time ***per week*** in total have you been prevented from carrying out such activities?

_____ **hours / days***
(*delete as appropriate)

12. Have other people carried out any of the unpaid activities for you? e.g shopping, odd jobs, chores, work in the household, or caring for others

(please circle) **Yes** **No**

***If Yes*:**
13. Please indicate who this was, how many hours they have helped and any costs incurred during the **last 3 months**. You may complete more than one box.

Type of help	**Number of hours**	**How much if anything did you have to pay for this help over the *last 3 months***
Family members (partner, children		
Other volunteer eg extended family, neighbours		
Help paid by social services		N/A
Help provided by the social services but paid for by you		
Other paid help		

EQ-5D

Health Questionnaire

By placing a tick in one box in each group below, please indicate which statements best describe your own health status today.

Mobility

I have no problems in walking about	❑
I have some problems in walking about	❑
I am confined to bed	❑

Self-Care

I have no problems with self-care	❑
I have some problems washing or dressing myself	❑
I am unable to wash or dress myself	❑

Usual Activities

I have no problems with performing my usual activities (e.g. work, study, housework, family or leisure activities)	❑
I have some problems with performing my usual activities	❑
I am unable to perform my usual activities	❑

Pain/Discomfort

I have no pain or discomfort	❑
I have moderate pain or discomfort	❑
I have extreme pain or discomfort	❑

Anxiety/Depression

I am not anxious or depressed	❑
I am moderately anxious or depressed	❑
I am extremely anxious or depressed	❑

Thank you for taking the time to complete these questions.

Mechanisms of tissue response to hyperbaric oxygen after radiotherapy

1 BACKGROUND

1.1 Late radiation injuries can be understood in terms of damage recognition and tissue remodelling

The traditional target cell model of late radiation adverse effects postulates irreversible and dose-dependent damage to the replicatory capacity of cells, leading to loss of parenchymal cells and vascular endothelium [120]. Arguments focus on the contribution of direct cell killing of parenchymal cells versus parenchymal atrophy secondary to vascular degeneration and tissue ischaemia [51]. The target cell model envisages radiation fibrosis as the passive stromal remnant of a tissue depleted of its cellular components. The target cell hypothesis remains a useful concept of early radiotherapy-induced injury, it has now become clear that it is an inadequate model of late injury, including progressive fibrosis. The so-called latent interval between radiation exposure and development of symptoms is not a quiescent phase at all but is characterised by a genetically regulated cytokine response mediated by a wide range of host cells, including inflammatory, stromal, endothelial and parenchymal components [14, 109]. An important element of this response involves activation of fibrogenic cytokines that deregulate extracellular matrix metabolism [70] leading to ongoing changes which can continue for decades. The significance of a genetically regulated stromal response is that it presents opportunities for intervention and modulation of molecular targets. Among a range of cell and molecular pathways that offer scope for investigation, the Rho/ROCK pathway is selected for further study as a key regulator of connective tissue growth factor expression and fibrogenesis in rectal submucosa..

1.2 The relationship between ischaemia and tissue fibrosis

Fibrosis is a prominent component of late radiotherapy change in the gastrointestinal tract [22, 68, 96, 107]. Hypoxia, mediated via a range of hypoxia-regulated genes, contributes to tissue scarring in other chronic fibrotic states. A close association has been noted between interstitial fibrosis and microvascular damage in progressive renal disease [89], where a sequence of events involving a heme-protein sensor is postulated with activation of hypoxia-inducible factor-1α (HIF-1α) [82, 100, 112, 138]. Several studies report upregulated *coll-i* gene expression and increased production of collagen-α1(i) in response to low oxygen tension [31, 119]. Downregulation of collagenase (MMP-1) and upregulation of metalloproteinase (TIMP-1) at mRNA and protein levels have also been reported.

After radiotherapy to the pelvis, there is convincing evidence that ischaemia is an early contributing factor to the pathological changes that occur within the gastrointestinal tract. Histologically, prominent vascular involvement has been noted in patients with early fibrotic change with endothelial cells separating from the basement membrane and blood vessel walls dilate with platelet clusters and thrombi in the vascular lumen. Subsequently, after some months abnormally proliferating endothelial cells contribute to vessel occlusion. Other studies show that these effects lead to sustained reduction in blood flow to the affected area [61, 77].

We have shown that ionizing radiation directly initiates TGF-β1 in skin fibroblasts and keratinocytes via activation of the AP-1 family transcription factors [38, 71]. In addition, local activation by ionising radiation of latent TGF-β1 trapped in extracellular matrix has been reported [29]. Interactions between TGF-β1 and extracellular matrix molecules

perpetuate chronic release of TGF-β and perpetuate activation of specific cell phenotypes, especially of myofibroblasts [11]. TGF-β1 is the primary fibrotic mediator that acts via activation of the smad 3/4 signaling pathway [32, 33, 69, 70, 103] and transactivation of α-smooth muscle actin and extracellular matrix genes [129-131]. However, recent studies suggest that the canonical TGF-b/ALK-5/Smad2-3 pathway does not mediate the fibrogenic signals in chronic and long-term established fibrotic lesions. More specifically, in irradiated human patients, we have also shown that fibrosis is associated with high levels of connective tissue growth factor (CTGF/CCN2) [133]. The molecular basis of elevated CTGF expression in human radiation enteropathy was studied using cDNA array profiling and classical biochemical approaches based on recombinant TGF-β1 and CTGF in primary smooth muscle cells and subepithelial myofibroblasts derived from affected patients. These cells maintain their fibrogenic features in long-term culture (6-8 passages), including altered contractile function, modification of the actin cytoskeleton and increased secretory activity. The cDNA approach showed activation of the Rho/ROCK pathway [132]. Functional in vitro experiments showed that this intercellular signaling pathway controlled CTGF expression in intestinal smooth muscles cells and subepithelial myofibroblasts [20]. The gene profiling suggested radiation enteropathy is associated with a global deregulation of stromal remodelling, with increased ECM deposition, elevated activity of metalloproteinases (MMP) and tissue inhibitors of metalloproteinases (TIMP) [116].

Cell response to increasing doses of recombinant TGF-β1 has also been investigated. Major activation of the Rho/ROCK pathway was detected in fibrosis-derived intestinal smooth muscles cells and subepithelial myofibroblasts, but the smad pathway was differentially activated in normal cells. These findings indicate differential fibrogenic response in normal versus fibrosis-derived cells, suggesting therapeutic opportunities for targeted anti-fibrotic therapies. The Rho GTPases regulate fundamental cellular processes including cell motility, cell cycle progression, cell survival, transcription, membrane trafficking and cytokinesis *via* their downstream effectors the Rho-associated kinase (ROCK) [105]. Many Rho functions have been elucidated using pharmacological inhibitors, the most prominent of these being statins that inhibit isoprenoid intermediates production and Rho activation. We showed that Rho/ROCK cascade regulates radiation-induced fibrogenic program in intestinal mesenchymal cells, pharmacological inhibition of Rho and ROCK activation were performed in vitro using pravastatin and Y-27632, a pyrimidine derivative inhibitor of ROCK. Both agents modulated the radiation-induced fibrogenic differentiation and the expression of CTGF, TGF-β1, and collagen Iα2 genes most likely *via* NF-κB inhibition [20, 46]. Local release of reactive oxygen species (ROS) has been observed in late radiation injury [134]. ROS alter intracellular signaling pathways, leading to transactivation of specific target genes such as those coding growth factors, cytokines, extracellular matrix compounds, and promoting oxidative inactivation of endothelial NO. The membrane associated NADH/NADPH oxidase enzyme complex appears to be the primary biochemical source of ROS in tissue, and NAD(P)H oxidase expression is known to be regulated by other members of Rho GTPases. Genetic and pharmacological (statin) inhibition of Rac and RhoA inhibits ROS production in vascular smooth muscle and endothelial cells [106]. The action of HBO on ROS release remains unclear, but the normalization of tissue oxygen gradient via neo-angiogenesis is postulated to be involved and might down-regulate hypoxia-inducible proteins. No direct link between hypoxia-inducible proteins and CTGF expression has been established to date. However, a recent study showed that chronic ischemic injury, but not acute injury, was associated with CTGF mRNA up-regulation [36].

Endoscopic biopsies of the human rectal mucosa, including a minimal amount of submucosa, can be collected under direct vision. The size of the mucosal deficit caused by the biopsies is 2-3 mm and is minimally traumatic even in patients with chronic proctopathy. The sites of such biopsies are macroscopically invisible within a short time. We have previously demonstrated that informative samples containing mucosa and some submucosa are easily obtained in this fashion. In preliminary experiments, we have established that two endoscopically directed biopsies taken from areas of the human rectum showing maximal change from prior radiotherapy supply adequate amounts of mRNA for further analysis.

2 AIMS

2.1 **Hypothesis:** therapeutic response to hyperbaric oxygen is associated with down-regulation of hypoxia-inducible proteins and the Rho/ROCK/CTGF pathway, and the reversal of activated cellular phenotypes responsible for progressive radiation fibrosis.

The aim is to collect, process and store tissue in order to test for (at a later date):

i) angiogenesis in irradiated rectal mucosa in response to HBO/sham therapy.
ii) down-regulation of the Rho/ROCK/CTGF pathway and other components of the fibrogenic cascade.
iii) association between angiogenesis and clinical response to HBO/sham therapy.

3 METHODS

3.1 **Hypothesis:** therapeutic response to hyperbaric oxygen is associated with down-regulation of hypoxia-inducible proteins and the Rho/ROCK/CTGF pathway, and the reversal of activated cellular phenotypes responsible for progressive radiation fibrosis.

3.1.1 Sample collection

Rectum: two triplicate rectal biopsies (six in total) will be collected via endoscopy before HBO or sham therapy in as many volunteers as possible (target is 80% of the whole group). Biopsies will be taken from the posterior third of the rectum, at 8 cm from the anal margin. The sites will be selected on the basis of visible mild to moderate radiation sequelae. The biopsies will aim to include mucosa and submucosa, but not muscle. Repeat samples will be collected from the same locations 2 weeks and 12 months after the completion of treatment.

3.1.2 Sample processing and storage

Two of each triplicate samples will be harvested and immediately snap frozen in liquid nitrogen for storage. For immunohistochemical studies, the second of each triplicate sample will be fixed in formalin, since many epitopes can be detected by conventional immunohistochemistry on paraffin sections.

3.1.3 Sample Analysis

This will form the basis of a future grant application once the clinical outcome data are known. At this point in time, investigations are likely to include the following:

Measurement of capillary density

Capillary density will be investigated using Chalkey point analysis [35]. Paraffin-fixed histological sections will be stained with CD34 antibody to demonstrate vascular endothelium. The Chalkey count is the number of grid points that hit stained microvessels, and is a relative area estimate rather than an absolute vessel count [128]. The means of the

three most vascular and least vascular areas within the submucosa (x200 magnification) will be estimated after manual selection of these areas by scanning the whole section at low power.

Immunohistochemistry for angiogenic and hypoxia-inducible factors
Standard methods for immunohistochemical assays will be applied to detect VEGF, HIF1α and other hypoxia-inducible proteins. Tissue distribution will be analysed in relation to the capillary network (see above).

Gene expression profiling
The future of research into the effects of radiation on normal tissues may well lie in the arena of gene expression profiling, using a stored tissue resource. It is generally recognized that expression of a number of genes is coordinated both spatially and temporally and that this coordination changes during the development and progression of late effects. Newly developed functional genomic approaches, such as serial analysis of gene expression (SAGE) and DNA microarrays have enabled researchers to determine the expression pattern of thousands of genes simultaneously [143]. Analyses will be undertaken of mRNA isolated from rectal wall biopsies.

3.1.4 Endpoints

Primary
Increase in blood vessel density from baseline (pre-treatment) to the end of HBO therapy (within 2 weeks of HBO therapy) will be correlated with therapeutic response i.e. reduction in bowel symptoms.
Secondary
Changes in proteins involved in extracellular matrix metabolism, including fibrogenic cytokines (including CTGF, TGFβ_1), collagen synthesis (including PINP, PIIINP, prolyl-4-hydroxylase) and metalloproteinases (including MMP-I).

4.0 STATISTICAL CONSIDERATIONS

4.1 Mechanisms of tissue healing following neoangiogensis induced by HBO in irradiated normal tissues

The sample size is determined by the number of research volunteers that will be randomised to receive 40 sessions of HBO (n=75), rather than by statistical considerations. Emphasis will be given to estimation rather than hypothesis testing. The mean increase in capillary density will be compared between randomised groups. In addition, correlations will be sought between change in capillary density and other endpoints, including immunohistochemical markers of neoangiogenesis, extracellular matrix metabolism and expression profiles. Differences between pre-and post-treatment samples are predicted in volunteers allocated HBO, but the analysis is most unlikely to take account of clinical response. The sham treated group will act as negative controls for these studies. If necessary, biomarker values will be transformed for analysis. For comparison of changes between the two trial arms, a standardized difference of 1 can be detected with 90% power, 1% significance level. As regards within patient change, a within patient standardised difference of 0.55 can be detected with 50 treated patients (alpha = 0.01 to allow for multiple comparisons, Power 90%) and a standardized difference of 0.8 can be detected in the 25 control patients. Control of the false discovery rate will be employed to minimize errors when assessing changes in mRNA expression. As regards correlations between change in capillary density and other endpoints, with 75 patients correlations of 0.45 or more can be reliably detected (90% power 1% significance level).

REFERENCES

1. Cancer Research UK. UK Incidence 1998. In: www.cancerresearchuk.org; 2002.
2. Epidermoid anal cancer: results from the UKCCCR randomised trial of radiotherapy alone versus radiotherapy, 5-fluorouracil, and mitomycin. UKCCCR Anal Cancer Trial Working Party. UK Co-ordinating Committee on Cancer Research. Lancet 1996;348(9034):1049-54.
3. Improved survival with preoperative radiotherapy in resectable rectal cancer. Swedish Rectal Cancer Trial. N Engl J Med 1997;336(14):980-7.
4. Aanderud L, Thorsen E, Brattebo G, Forland M, Kristensen G. [Hyperbaric oxygen treatment for radiation reactions]. Tidsskr Nor Laegeforen 2000;120(9):1020-2.
5. Aicher A, Heeschen C, Mildner-Rihm C, Urbich C, Ihling C, Technau-Ihling K, Zeiher AM, Dimmeler S. Essential role of endothelial nitric oxide synthase for mobilization of stem and progenitor cells. Nat Med 2003;9(11):1370-6.
6. al-Abany M, Helgason AR, Cronqvist AK, Svensson C, Wersall P, Steineck G. Long-term symptoms after external beam radiation therapy for prostate cancer with three or four fields. Acta Oncol 2002;41(6):532-42.
7. Andreyev H, Vlavianos P, Blake P, Dearnaley D, Norman A, Tait D. Gastrointestinal symptoms after pelvic radiotherapy: is there any role for the gastroenterologist? Int J Rad Oncol Biol Phys 2005;62(5):1464-1471.
8. Andreyev J. Gastrointestinal complications of pelvic radiotherapy: are they of any importance? Gut 2005;54(8):1051-4.
9. Asahara T, Takahashi T, Masuda H, Kalka C, Chen D, Iwaguro H, Inai Y, Silver M, Isner JM. VEGF contributes to postnatal neovascularization by mobilizing bone marrow-derived endothelial progenitor cells. Embo J 1999;18(14):3964-72.
10. Bacon CG, Giovannucci E, Testa M, Glass TA, Kawachi I. The association of treatment-related symptoms with quality-of-life outcomes for localized prostate carcinoma patients. Cancer 2002;94(3):862-71.
11. Barcellos-Hoff MH, Costes SV. A systems biology approach to multicellular and multi-generational radiation responses. Mutat Res 2006;597(1-2):32-8.
12. Bauer SM, Goldstein LJ, Bauer RJ, Chen H, Putt M, Velazquez OC. The bone marrow-derived endothelial progenitor cell response is impaired in delayed wound healing from ischemia. J Vasc Surg 2006;43(1):134-41.
13. Bem J, Bem S, Singh A. Use of hyperbaric oxygen chamber in the management of radiation-related complications of the anorectal region: report of two cases and review of the literature. Dis Colon Rectum 2000;43(10):1435-8.
14. Bentzen SM. Preventing or reducing late side effects of radiation therapy: radiobiology meets molecular pathology. Nat Rev Cancer 2006;6(9):702-13.
15. Bergmark K, Avall-Lundqvist E, Dickman PW, Henningsohn L, Steineck G. Patient-rating of distressful symptoms after treatment for early cervical cancer. Acta Obstet Gynecol Scand 2002;81(5):443-50.
16. Berthrong M, Fajardo LF. Radiation injury in surgical pathology. Part II. Alimentary tract. Am J Surg Pathol 1981;5(2):153-78.
17. Bevers RF, Bakker DJ, Kurth KH. Hyperbaric oxygen treatment for haemorrhagic radiation cystitis. Lancet 1995;346(8978):803-5.
18. Boersma LJ, van den Brink M, Bruce AM, Shouman T, Gras L, te Velde A, Lebesque JV. Estimation of the incidence of late bladder and rectum complications after high-dose (70-78 GY) conformal radiotherapy for prostate cancer, using dose-volume histograms. Int J Radiat Oncol Biol Phys 1998;41(1):83-92.
19. Borghede G, Hedelin H. Radiotherapy of localised prostate cancer. Analysis of late treatment complications. Radiother Oncol 1997;43(2):139-46.
20. Bourgier C, Haydont V, Milliat F, Francois A, Holler V, Lasser P, Bourhis J, Mathe D, Vozenin-Brotons MC. Inhibition of Rho kinase modulates radiation induced fibrogenic phenotype in intestinal smooth muscle cells through alteration of the cytoskeleton and connective tissue growth factor expression. Gut 2005;54(3):336-43.

21. Clarke D, Tenorio C, Toklu A, Desai S, Hussey J, Cone L. Treatment of radiation necrosis with HBO: a randomized, double-blind, placebo controlled trial. In. The Undersea & Hyperbaric Medicine Society's Annual Scientific Meeting, Sydney, Australia 2004; 2004.
22. Coia LR, Myerson RJ, Tepper JE. Late effects of radiation therapy on the gastrointestinal tract. Int J Radiat Oncol Biol Phys 1995;31(5):1213-36.
23. Dahlberg M, Glimelius B, Graf W, Pahlman L. Preoperative irradiation affects functional results after surgery for rectal cancer: results from a randomized study. Dis Colon Rectum 1998;41(5):543-9; discussion 549-51.
24. Dall'Era MA, Hampson NB, Hsi RA, Madsen B, Corman JM. Hyperbaric oxygen therapy for radiation induced proctopathy in men treated for prostate cancer. J Urol 2006;176(1):87-90.
25. Dearnaley DP, Khoo VS, Norman AR, Meyer L, Nahum A, Tait D, Yarnold J, Horwich A. Comparison of radiation side-effects of conformal and conventional radiotherapy in prostate cancer: a randomised trial. Lancet 1999;353(9149):267-72.
26. Denton AS, Andreyev HJ, Forbes A, Maher EJ. Systematic review for non-surgical interventions for the management of late radiation proctitis. Br J Cancer 2002;87(2):134-43.
27. Denton AS, Bond SJ, Matthews S, Bentzen SM, Maher EJ. National audit of the management and outcome of carcinoma of the cervix treated with radiotherapy in 1993. Clin Oncol (R Coll Radiol) 2000;12(6):347-53.
28. Douglass HO, Jr., Moertel CG, Mayer RJ, Thomas PR, Lindblad AS, Mittleman A, Stablein DM, Bruckner HW. Survival after postoperative combination treatment of rectal cancer. N Engl J Med 1986;315(20):1294-5.
29. Ehrhart EJ, Segarini P, Tsang ML, Carroll AG, Barcellos-Hoff MH. Latent transforming growth factor beta1 activation in situ: quantitative and functional evidence after low-dose gamma-irradiation. Faseb J 1997;11(12):991-1002.
30. Eifel PJ, Levenback C, Wharton JT, Oswald MJ. Time course and incidence of late complications in patients treated with radiation therapy for FIGO stage IB carcinoma of the uterine cervix. Int J Radiat Oncol Biol Phys 1995;32(5):1289-300.
31. Falanga V, Zhou L, Yufit T. Low oxygen tension stimulates collagen synthesis and COL1A1 transcription through the action of TGF-beta1. J Cell Physiol 2002;191(1):42-50.
32. Flanders KC, Major CD, Arabshahi A, Aburime EE, Okada MH, Fujii M, Blalock TD, Schultz GS, Sowers A, Anzano MA, Mitchell JB, Russo A, Roberts AB. Interference with transforming growth factor-beta/ Smad3 signaling results in accelerated healing of wounds in previously irradiated skin. Am J Pathol 2003;163(6):2247-57.
33. Flanders KC, Sullivan CD, Fujii M, Sowers A, Anzano MA, Arabshahi A, Major C, Deng C, Russo A, Mitchell JB, Roberts AB. Mice lacking Smad3 are protected against cutaneous injury induced by ionizing radiation. Am J Pathol 2002;160(3):1057-68.
34. Fokdal L, Hoyer M, Meldgaard P, von der Maase H. Long-term bladder, colorectal, and sexual functions after radical radiotherapy for urinary bladder cancer. Radiother Oncol 2004;72(2):139-45.
35. Fox SB, Leek RD, Weekes MP, Whitehouse RM, Gatter KC, Harris AL. Quantitation and prognostic value of breast cancer angiogenesis: comparison of microvessel density, Chalkley count, and computer image analysis. J Pathol 1995;177(3):275-83.
36. Gabrielsen A, Lawler PR, Yongzhong W, Steinbruchel D, Blagoja D, Paulsson-Berne G, Kastrup J, Hansson GK. Gene expression signals involved in ischemic injury, extracellular matrix composition and fibrosis defined by global mRNA profiling of the human left ventricular myocardium. J Mol Cell Cardiol 2007.
37. Gami B, Harrington K, Blake P, Dearnaley D, Tait D, Davies J, Norman AR, Andreyev HJ. How patients manage gastrointestinal symptoms after pelvic radiotherapy. Aliment Pharmacol Ther 2003;18(10):987-94.
38. Gault N, Vozenin-Brotons MC, Calenda A, Lefaix JL, Martin MT. Promoter sequences involved in transforming growth factor beta1 gene induction in HaCaT keratinocytes after gamma irradiation. Radiat Res 2002;157(3):249-55.
39. Gelber RD, Goldhirsch A, Cole BF, Wieand HS, Schroeder G, Krook JE. A quality-adjusted time without symptoms or toxicity (Q-TWiST) analysis of adjuvant radiation therapy and chemotherapy for resectable rectal cancer. J Natl Cancer Inst 1996;88(15):1039-45.
40. Gouello JP, Bouachour G, Person B, Ronceray J, Cellier P, Alquier P. [The role of hyperbaric oxygen therapy in radiation-induced digestive disorders. 36 cases]. Presse Med 1999;28(20):1053-7.

41. Grim PS, Gottlieb LJ, Boddie A, Batson E. Hyperbaric oxygen therapy. Jama 1990;263(16):2216-20.
42. Haddock MG, Martenson JA. Anal carcinoma - anal function. In: Clinical Radiation Oncology. New York: Churchill Livingstone; 2000. p. 754.
43. Hamour AA, Denning DW. Hyperbaric oxygen therapy in a woman who declined colostomy. Lancet 1996;348(9021):197.
44. Harmon FW, Marx RE. The effect of hyperbaric oxygen on carcinogenesis and tumor growth rate. In. 65th Annual Meeting of the American Association of Oral and Maxillofacial Surgeons; 1983.
45. Hauer-Jensen M, Wang J, Denham JW. Bowel injury: current and evolving management strategies. Semin Radiat Oncol 2003;13(3):357-71.
46. Haydont V, Mathe D, Bourgier C, Abdelali J, Aigueperse J, Bourhis J, Vozenin-Brotons MC. Induction of CTGF by TGF-beta1 in normal and radiation enteritis human smooth muscle cells: Smad/Rho balance and therapeutic perspectives. Radiother Oncol 2005;76(2):219-25.
47. Heissig B, Hattori K, Dias S, Friedrich M, Ferris B, Hackett NR, Crystal RG, Besmer P, Lyden D, Moore MA, Werb Z, Rafii S. Recruitment of stem and progenitor cells from the bone marrow niche requires MMP-9 mediated release of kit-ligand. Cell 2002;109(5):625-37.
48. Henningsohn L, Wijkstrom H, Dickman PW, Bergmark K, Steineck G. Distressful symptoms after radical radiotherapy for urinary bladder cancer. Radiother Oncol 2002;62(2):215-25.
49. Hlavaty T, Persoons P, Vermeire S, Ferrante M, Pierik M, Van Assche G, Rutgeerts P. Evaluation of short-term responsiveness and cutoff values of inflammatory bowel disease questionnaire in Crohn's disease. Inflamm Bowel Dis 2006;12(3):199-204.
50. Hopewell J, Withers HR. Proposition: long-term changes in irradiated tissues are due principally to vascular damage in the tissues. Med Phys 1998;25(12):2265-8.
51. Hopewell JW, Calvo W, Jaenke R, Reinhold HS, Robbins ME, Whitehouse EM. Microvasculature and radiation damage. Recent Results Cancer Res 1993;130:1-16.
52. Hopewell JW, Young CM. Changes in the microcirculation of normal tissues after irradiation. Int J Radiat Oncol Biol Phys 1978;4(1-2):53-8.
53. Kang TS, Gorti GK, Quan SY, Ho M, Koch RJ. Effect of hyperbaric oxygen on the growth factor profile of fibroblasts. Arch Facial Plast Surg 2004;6(1):31-5.
54. Karakurum M, Shreeniwas R, Chen J, Pinsky D, Yan SD, Anderson M, Sunouchi K, Major J, Hamilton T, Kuwabara K, et al. Hypoxic induction of interleukin-8 gene expression in human endothelial cells. J Clin Invest 1994;93(4):1564-70.
55. Khalid U, McGough C, Hackett C, Blake P, Harrington K, Khoo V, Tait D, Norman A, Andreyev H. A modified inflammatory bowel disease questionnaire and the Vaizey Incontinence questionnaire are more sensitive measures of acute gastrointestinal toxicity during pelvic radiotherapy than RTOG grading. Int J Radiat Oncol Biol Phys 2006;64(5):1432-41.
56. Kindwall EP. Hyperbaric oxygen treatment of radiation cystitis. Clin Plast Surg 1993;20(3):589-92.
57. Kitta T, Shinohara N, Shirato H, Otsuka H, Koyanagi T. The treatment of chronic radiation proctitis with hyperbaric oxygen in patients with prostate cancer. BJU Int 2000;85(3):372-4.
58. Knighton DR, Hunt TK, Scheuenstuhl H, Halliday BJ, Werb Z, Banda MJ. Oxygen tension regulates the expression of angiogenesis factor by macrophages. Science 1983;221(4617):1283-5.
59. Knighton DR, Silver IA, Hunt TK. Regulation of wound-healing angiogenesis-effect of oxygen gradients and inspired oxygen concentration. Surgery 1981;90(2):262-70.
60. Kollmorgen CF, Meagher AP, Wolff BG, Pemberton JH, Martenson JA, Illstrup DM. The long-term effect of adjuvant postoperative chemoradiotherapy for rectal carcinoma on bowel function. Ann Surg 1994;220(5):676-82.
61. Konishi T, Watanabe T, Kitayama J, Shibahara J, Nagawa H. Endoscopic and histopathologic findings after formalin application for hemorrhage caused by chronic radiation-induced proctitis. Gastrointest Endosc 2005;61(1):161-4.
62. Kozelsky TF, Meyers GE, Sloan JA, Shanahan TG, Dick SJ, Moore RL, Engeler GP, Frank AR, McKone TK, Urias RE, Pilepich MV, Novotny PJ, Martenson JA. Phase III double-blind study of glutamine versus placebo for the prevention of acute diarrhea in patients receiving pelvic radiation therapy. J Clin Oncol 2003;21(9):1669-74.
63. Kupelian PA, Thakkar VV, Khuntia D, Reddy CA, Klein EA, Mahadevan A. Hypofractionated intensity-modulated radiotherapy (70 gy at 2.5 Gy per fraction) for localized prostate cancer: long-term outcomes. Int J Radiat Oncol Biol Phys 2005;63(5):1463-8.

64. Lee HC, Liu CS, Chiao C, Lin SN. Hyperbaric oxygen therapy in hemorrhagic radiation cystitis: a report of 20 cases. Undersea Hyperb Med 1994;21(3):321-7.
65. Lillemoe KD, Brigham RA, Harmon JW, Feaster MM, Saunders JR, d'Avis JA. Surgical management of small-bowel radiation enteritis. Arch Surg 1983;118(8):905-7.
66. Lin S, Shyu KG, Lee CC, Wang BW, Chang CC, Liu YC, Huang FY, Chang H. Hyperbaric oxygen selectively induces angiopoietin-2 in human umbilical vein endothelial cells. Biochem Biophys Res Commun 2002;296(3):710-5.
67. Lundby L, Jensen VJ, Overgaard J, Laurberg S. Long-term colorectal function after postoperative radiotherapy for colorectal cancer. Lancet 1997;350(9077):564.
68. Marks LB, Carroll PR, Dugan TC, Anscher MS. The response of the urinary bladder, urethra, and ureter to radiation and chemotherapy. Int J Radiat Oncol Biol Phys 1995;31(5):1257-80.
69. Martin M, Delanian S, Sivan V, Vozenin-Brotons MC, Reisdorf P, Lawrence D, Lefaix JL. [Radiation-induced superficial fibrosis and TGF-alpha 1]. Cancer Radiother 2000;4(5):369-84.
70. Martin M, Lefaix J, Delanian S. TGF-beta1 and radiation fibrosis: a master switch and a specific therapeutic target? Int J Radiat Oncol Biol Phys 2000;47(2):277-90.
71. Martin M, Vozenin MC, Gault N, Crechet F, Pfarr CM, Lefaix JL. Coactivation of AP-1 activity and TGF-beta1 gene expression in the stress response of normal skin cells to ionizing radiation. Oncogene 1997;15(8):981-9.
72. Marx RE. Hyperbaric Medicine Practice: Chapter 23, Radiation Injury to Tissue. In: Hyperbaric Medicine Practice. Second ed: Best Publishing Company; 1994. p. 448-501.
73. Marx RE, Ehler WJ, Tayapongsak P, Pierce LW. Relationship of oxygen dose to angiogenesis induction in irradiated tissue. Am J Surg 1990;160(5):519-24.
74. Marx RE, Johnson RP. Problem wounds in oral and maxillofacial surgery: the role of hyperbarix oxygen. In: Davis JC, Hunt TK, editors. Problem Wounds - The role of oxygen. New York: Elsevier; 1988. p. 65-123.
75. Marx RE, Johnson RP, Kline SN. Prevention of osteoradionecrosis: a randomized prospective clinical trial of hyperbaric oxygen versus penicillin. J Am Dent Assoc 1985;111(1):49-54.
76. Michalski JM, Purdy JA, Winter K, Roach M, 3rd, Vijayakumar S, Sandler HM, Markoe AM, Ritter MA, Russell KJ, Sailer S, Harms WB, Perez CA, Wilder RB, Hanks GE, Cox JD. Preliminary report of toxicity following 3D radiation therapy for prostate cancer on 3DOG/RTOG 9406. Int J Radiat Oncol Biol Phys 2000;46(2):391-402.
77. Milsom JW, Senagore A, Walshaw RK, Mostosky UV, Wang P, Johnson W, Chaudry IH. Preoperative radiation therapy produces an early and persistent reduction in colorectal anastomotic blood flow. J Surg Res 1992;53(5):464-9.
78. Minchenko A, Salceda S, Bauer T, Caro J. Hypoxia regulatory elements of the human vascular endothelial growth factor gene. Cell Mol Biol Res 1994;40(1):35-9.
79. Moore EM, Magrino TJ, Johnstone PA. Rectal bleeding after radiation therapy for prostate cancer: endoscopic evaluation. Radiology 2000;217(1):215-8.
80. Morris AD, Zeitman AL, Kaufman DS, Shipley WU. Bladder cancer - muscle invading bladder cancer. In: Clinical Radiation Oncology. New York; 2000. p. 825-35.
81. Morris M, Eifel PJ, Lu J, Grigsby PW, Levenback C, Stevens RE, Rotman M, Gershenson DM, Mutch DG. Pelvic radiation with concurrent chemotherapy compared with pelvic and para-aortic radiation for high-risk cervical cancer. N Engl J Med 1999;340(15):1137-43.
82. Morwenna S, Ratcliffe WP. Mammalian oxygen sensing and hypoxia inducible factor-1. Int J Biochem Cell Biol 1997;29(12):1419-32.
83. Moulin C, Li V, Loizzo F, Faudemay C, Hostein J. [Value of hyperbaric oxygen in the hemostatic treatment of chronic radiation-induced recto-sigmoiditis]. Gastroenterol Clin Biol 1993;17(6-7):520-1.
84. Nakada T, Kubota Y, Sasagawa I, Suzuki H, Yamaguchi T, Ishigooka M, Kakizaki H. Therapeutic experience of hyperbaric oxygenation in radiation colitis. Report of a case. Dis Colon Rectum 1993;36(10):962-5.
85. Nakamura Y, Tajima F, Ishiga K, Yamazaki H, Oshimura M, Shiota G, Murawaki Y. Soluble c-kit receptor mobilizes hematopoietic stem cells to peripheral blood in mice. Exp Hematol 2004;32(4):390-6.
86. Nemiroff PM. Effects of hyperbaric oxygen on tumour metastases in mice. Clin Radiol 1991;22:538-540.

87. Neurath MF, Branbrink A, Meyer zum Buschenfelde KH, Lohse AW. A new treatment for severe malabsorption due to radiation enteritis. Lancet 1996;347(9011):1302.
88. Norkool DM, Hampson NB, Gibbons RP, Weissman RM. Hyperbaric oxygen therapy for radiation-induced hemorrhagic cystitis. J Urol 1993;150(2 Pt 1):332-4.
89. Norman JT, Clark IM, Garcia PL. Hypoxia promotes fibrogenesis in human renal fibroblasts. Kidney Int 2000;58(6):2351-66.
90. O'Brien PC, Hamilton CS, Denham JW, Gourlay R, Franklin CI. Spontaneous improvement in late rectal mucosal changes after radiotherapy for prostate cancer. Int J Radiat Oncol Biol Phys 2004;58(1):75-80.
91. O'Brien PH, Jenrette JM, 3rd, Garvin AJ. Radiation enteritis. Am Surg 1987;53(9):501-4.
92. Olopade F, Norman A, Blake P, Dearnaley D, Harrington K, Khoo V, Tait D, Hackett C, Andreyev H. A modified Inflammatory Bowel Disease questionnaire and the Vaizey Incontinence questionnaire are simple ways to identify patients with significant gastrointestinal symptoms after pelvic radiotherapy. Br J Cancer 2005;92(9):1663-70.
93. Ooi BS, Tjandra JJ, Green MD. Morbidities of adjuvant chemotherapy and radiotherapy for resectable rectal cancer: an overview. Dis Colon Rectum 1999;42(3):403-18.
94. Patel B, Khaliq A, Jarvis-Evans J, McLeod D, Mackness M, Boulton M. Oxygen regulation of TGF-beta 1 mRNA in human hepatoma (Hep G2) cells. Biochem Mol Biol Int 1994;34(3):639-44.
95. Pavy JJ, Denekamp J, Letschert J, Littbrand B, Mornex F, Bernier J, Gonzales-Gonzales D, Horiot JC, Bolla M, Bartelink H. EORTC Late Effects Working Group. Late effects toxicity scoring: the SOMA scale. Radiother Oncol 1995;35(1):11-5.
96. Perez CA, Fox S, Lockett MA, Grigsby PW, Camel HM, Galakatos A, Kao MS, Williamson J. Impact of dose in outcome of irradiation alone in carcinoma of the uterine cervix: analysis of two different methods. Int J Radiat Oncol Biol Phys 1991;21(4):885-98.
97. Peterson IA, Viria MA. Endometrial cancer - definitive irradiation - indications and results. In: Clinical Radiation Oncology. New York: Churchill Livingstone; 2000. p. 911-13.
98. Pisansky TM, Lee WR, Sawton CA, Bostwick DG. Prostate cancer - radiation therapy, general principles. In: Clinical Radiation Oncology. New York: Churchill Livingstone; 2000. p. 782-83.
99. Rafii S, Lyden D. Therapeutic stem and progenitor cell transplantation for organ vascularization and regeneration. Nat Med 2003;9(6):702-12.
100. Ratcliffe PJ. Molecular biology of erythropoietin. Kidney Int 1993;44(4):887-904.
101. Rehman J, Li J, Parvathaneni L, Karlsson G, Panchal VR, Temm CJ, Mahenthiran J, March KL. Exercise acutely increases circulating endothelial progenitor cells and monocyte-/macrophage-derived angiogenic cells. J Am Coll Cardiol 2004;43(12):2314-8.
102. Reichelderfer M, Morrissey JF. Colonoscopy in radiation colitis. Gastrointest Endosc 1980;26(2):41-3.
103. Reisdorf P, Lawrence DA, Sivan V, Klising E, Martin MT. Alteration of transforming growth factor-beta1 response involves down-regulation of Smad3 signaling in myofibroblasts from skin fibrosis. Am J Pathol 2001;159(1):263-72.
104. Reyes M, Dudek A, Jahagirdar B, Koodie L, Marker PH, Verfaillie CM. Origin of endothelial progenitors in human postnatal bone marrow. J Clin Invest 2002;109(3):337-46.
105. Ridley AJ. Rho family proteins: coordinating cell responses. Trends Cell Biol 2001;11(12):471-7.
106. Rolfe BE, Worth NF, World CJ, Campbell JH, Campbell GR. Rho and vascular disease. Atherosclerosis 2005;183(1):1-16.
107. Rubin P, Casarett GW. Clinical Radiation Pathology. Philadelphia: WB Saunders Company; 1968.
108. Rubin P, Constine LS, Fajardo LF, Phillips TL, Wasserman TH. RTOG Late Effects Working Group. Overview. Late Effects of Normal Tissues (LENT) scoring system. Int J Radiat Oncol Biol Phys 1995;31(5):1041-2.
109. Rubin P, Johnston CJ, Williams JP, McDonald S, Finkelstein JN. A perpetual cascade of cytokines postirradiation leads to pulmonary fibrosis. Int J Radiat Oncol Biol Phys 1995;33(1):99-109.
110. Scannell G, Waxman K, Kaml GJ, Ioli G, Gatanaga T, Yamamoto R, Granger GA. Hypoxia induces a human macrophage cell line to release tumor necrosis factor-alpha and its soluble receptors in vitro. J Surg Res 1993;54(4):281-5.
111. Schoenrock GJ, Cianci P. Treatment of radiation cystitis with hyperbaric oxygen. Urology 1986;27(3):271-2.

112. Semenza GL. Hypoxia-inducible factor 1 and the molecular physiology of oxygen homeostasis. J Lab Clin Med 1998;131(3):207-14.
113. Sheikh AY, Gibson JJ, Rollins MD, Hopf HW, Hussain Z, Hunt TK. Effect of hyperoxia on vascular endothelial growth factor levels in a wound model. Arch Surg 2000;135(11):1293-7.
114. Shintani S, Murohara T, Ikeda H, Ueno T, Honma T, Katoh A, Sasaki K, Shimada T, Oike Y, Imaizumi T. Mobilization of endothelial progenitor cells in patients with acute myocardial infarction. Circulation 2001;103(23):2776-9.
115. Silver I. Cellular Microenvironment in Healing and Non-Healing Wounds. 1984(4):50-66.
116. Strup-Perrot C, Mathe D, Linard C, Violot D, Milliat F, Francois A, Bourhis J, Vozenin-Brotons MC. Global gene expression profiles reveal an increase in mRNA levels of collagens, MMPs, and TIMPs in late radiation enteritis. Am J Physiol Gastrointest Liver Physiol 2004;287(4):G875-85.
117. Stryker JA, Bartholomew M. Failure of lactose-restricted diets to prevent radiation-induced diarrhea in patients undergoing whole pelvis irradiation. Int J Radiat Oncol Biol Phys 1986;12(5):789-92.
118. Takahashi T, Kalka C, Masuda H, Chen D, Silver M, Kearney M, Magner M, Isner JM, Asahara T. Ischemia- and cytokine-induced mobilization of bone marrow-derived endothelial progenitor cells for neovascularization. Nat Med 1999;5(4):434-8.
119. Tamamori M, Ito H, Hiroe M, Marumo F, Hata RI. Stimulation of collagen synthesis in rat cardiac fibroblasts by exposure to hypoxic culture conditions and suppression of the effect by natriuretic peptides. Cell Biol Int 1997;21(3):175-80.
120. Thames HD, Hendry JH. Fractionation in radiotherapy. London: Taylor and Frnacis; 1987.
121. Thom SR, Bhopale V, Fisher D, Manevich Y, Huang PL, Buerk DG. Stimulation of nitric oxide synthase in cerebral cortex due to elevated partial pressures of oxygen: an oxidative stress response. J Neurobiol 2002;51(2):85-100.
122. Thom SR, Bhopale VM, Velazquez OC, Goldstein LJ, Thom LH, Buerk DG. Stem Cell Mobilization by Hyperbaric Oxygen. Am J Phsiol Heart Circ Physiol 2006 Apr;290(4):H1378-86.
123. Thomas GM, Murphy KJ. Carcinoma of the vagina. In: Clinical Radiation Oncology. New York; 2000. p. 932-35.
124. Thomas GM, Murphy KJ. Vulvar carcinoma - radiation therapy. In: Clinical Radiation Oncology. New York: Churchill Livingstone; 2000. p. 924-31.
125. To LB, Haylock DN, Simmons PJ, Juttner CA. The biology and clinical uses of blood stem cells. Blood 1997;89(7):2233-58.
126. Tong J, Hoffman R, Siena S, Srour EF, Bregni M, Gianni AM. Characterization and quantitation of primitive hematopoietic progenitor cells present in peripheral blood autografts. Exp Hematol 1994;22(10):1016-24.
127. Turesson I. The progression rate of late radiation effects in normal tissue and its impact on dose-response relationships. Radiother Oncol 1989;15(3):217-26.
128. Vermeulen PB, Gasparini G, Fox SB, Colpaert C, Marson LP, Gion M, Belien JA, de Waal RM, Van Marck E, Magnani E, Weidner N, Harris AL, Dirix LY. Second international consensus on the methodology and criteria of evaluation of angiogenesis quantification in solid human tumours. Eur J Cancer 2002;38(12):1564-79.
129. Verrecchia F, Chu ML, Mauviel A. Identification of novel TGF-beta /Smad gene targets in dermal fibroblasts using a combined cDNA microarray/promoter transactivation approach. J Biol Chem 2001;276(20):17058-62.
130. Verrecchia F, Mauviel A. Control of connective tissue gene expression by TGF beta: role of Smad proteins in fibrosis. Curr Rheumatol Rep 2002;4(2):143-9.
131. Verrecchia F, Mauviel A. Transforming growth factor-beta signaling through the Smad pathway: role in extracellular matrix gene expression and regulation. J Invest Dermatol 2002;118(2):211-5.
132. Vozenin-Brotons MC, Milliat F, Linard C, Strup C, Francois A, Sabourin JC, Lasser P, Lusinchi A, Deutsch E, Girinsky T, Aigueperse J, Bourhis J, Mathe D. Gene expression profile in human late radiation enteritis obtained by high-density cDNA array hybridization. Radiat Res 2004;161(3):299-311.
133. Vozenin-Brotons MC, Milliat F, Sabourin JC, de Gouville AC, Francois A, Lasser P, Morice P, Haie-Meder C, Lusinchi A, Antoun S, Bourhis J, Mathe D, Girinsky T, Aigueperse J. Fibrogenic signals in patients with radiation enteritis are associated with increased connective tissue growth factor expression. Int J Radiat Oncol Biol Phys 2003;56(2):561-72.

134. Vujaskovic Z, Anscher MS, Feng QF, Rabbani ZN, Amin K, Samulski TS, Dewhirst MW, Haroon ZA. Radiation-induced hypoxia may perpetuate late normal tissue injury. Int J Radiat Oncol Biol Phys 2001;50(4):851-5.
135. Wachter S, Gerstner N, Goldner G, Potzi R, Wambersie A, Potter R. Endoscopic scoring of late rectal mucosal damage after conformal radiotherapy for prostatic carcinoma. Radiother Oncol 2000;54(1):11-9.
136. Warren DC, Feehan P, Slade JB, Cianci PE. Chronic radiation proctitis treated with hyperbaric oxygen. Undersea Hyperb Med 1997;24(3):181-4.
137. Weiss JP, Mattei DM, Neville EC, Hanno PM. Primary treatment of radiation-induced hemorrhagic cystitis with hyperbaric oxygen: 10-year experience. J Urol 1994;151(6):1514-7.
138. Wenger RH, Gassmann M. Oxygen(es) and the hypoxia-inducible factor-1. Biol Chem 1997;378(7):609-16.
139. Willett CG, Fung CY, Kaufman DS, Efird J, Shellito PC. Postoperative radiation therapy for high-risk colon carcinoma. J Clin Oncol 1993;11(6):1112-7.
140. Williams H, Vlavianos P, Blake P, Dearnaley D, Tait D, Andreyev H. The significance of rectal bleeding after pelvic radiotherapy. Radiother Oncol 2004;73(Suppl 1):S378.
141. Wobbes T, Verschueren RC, Lubbers EJ, Jansen W, Paping RH. Surgical aspects of radiation enteritis of the small bowel. Dis Colon Rectum 1984;27(2):89-92.
142. Woo TC, Joseph D, Oxer H. Hyperbaric oxygen treatment for radiation proctitis. Int J Radiat Oncol Biol Phys 1997;38(3):619-22.
143. Ye SQ, Usher DC, Zhang LQ. Gene expression profiling of human diseases by serial analysis of gene expression. J Biomed Sci 2002;9(5):384-94.
144. Yeoh EE, Botten R, Russo A, McGowan R, Fraser R, Roos D, Penniment M, Borg M, Sun W. Chronic effects of therapeutic irradiation for localized prostatic carcinoma on anorectal function. Int J Radiat Oncol Biol Phys 2000;47(4):915-24.
145. Zelefsky MJ, Cowen D, Fuks Z, Shike M, Burman C, Jackson A, Venkatramen ES, Leibel SA. Long term tolerance of high dose three-dimensional conformal radiotherapy in patients with localized prostate carcinoma. Cancer 1999;85(11):2460-8.