Article type: Original article Word count: 3398 (max 3500)

Title: Hypofractionated radiotherapy in invasive bladder cancer: an individual patient data meta-analysis of the BC2001 and BCON trials

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Key words (maximum of six key words): muscle invasive bladder cancer, radiotherapy, hypofractionation, chemotherapy, BC2001 trial, BCON trial, hypoxia

Presented at the 2019 ASTRO Annual Meeting

Research in context

Evidence before this study

Before this individual patient data (IPD) meta-analysis was initiated (January 2019), to our knowledge there were no published randomised controlled trials or meta-analyses comparing the two most common dose and fraction schedules used in muscle-invasive bladder cancer. Both 64 Gy in 32 fractions and 55 Gy in 20 fractions are used as standard treatment in the UK and were part of the protocols for both the BC2001 and BCON randomised controlled trials. We searched PubMed using the terms (hypofractionated radiotherapy AND muscle-invasive bladder cancer) AND (loco-regional control)) AND (overall survival)) for clinical trials and meta-analyses published up to 31 May 2020. We identified zero studies directly comparing the two schedules, although published series suggested that outcome and late toxicity were comparable.

Added value of this study

To our knowledge, this is the first published individual patient data meta-analysis comparing the outcomes from the two most commonly used radiotherapy schedules for muscle-invasive bladder cancer. This study aimed to confirm that moderately hypofractionated radiotherapy with 55 Gy in 20 fractions over 4 weeks was non-inferior to 64 Gy in 32 fractions over 6.5 weeks for invasive loco-regional control (ILRC) at 5 years. This study provides compelling evidence that moderately hypofractionated radiotherapy is not only non-inferior, but significantly improves ILRC rate regardless of radiosensitisation or radiosensitiser.

Implications of all the available evidence

With these findings, 55 Gy in 20 fractions over 4 weeks should be the new standard of care for patients undergoing bladder preservation for muscle-invasive bladder cancer.

Abstract (max 300 words, current: 300)

Background

Two radiotherapy fractionation schedules are used for treating muscle invasive bladder cancer (MIBC): 64Gy in 32 fractions (f) over 6.5-weeks and a hypofractionated schedule of 55Gy in 20f over 4-weeks. Long-term outcomes from several studies suggest that response, survival and toxicity are comparable, but there is no direct comparison published. This work aimed to assess non-inferiority (NI) of 55Gy/20f to 64Gy/32f in terms of invasive loco-regional control (ILRC), and late toxicity in MIBC patients.

Methods

Individual patient data (IPD) for patients with invasive BC (T1G3, T2-T4, N0, M0) were obtained from two multicentre randomised controlled trials: BC2001 (NCT00024349), assessing addition of chemotherapy to radiotherapy, and BCON (NCT00033436), which investigated combining hypoxia-modifying therapy with radiotherapy. In both trials, fractionation schedule was according to local standard practice. Co-primary endpoints were ILRC, rate free of muscle-invasive bladder recurrence or recurrence in pelvic nodes (pre-specified NI margin hazard ratio (HR) 1.25); and late bladder/rectal toxicity, assessed by LENT/SOMA (pre-specified NI margin for absolute risk difference (RD) 10%) . One-stage IPD meta-analysis models for time-to-event and binary outcomes were used, accounting for trial differences, within-centre correlation, randomised treatment received, baseline imbalances and potential confounding from relevant prognostic factors.

Findings

782 patients (456 BC2001, 326 BCON; 376 64Gy/32f, 406 55Gy/20f) were included (mean age 72 years, 80% stage T1/2); median follow-up was 120 months. Patients receiving 55Gy/20f had 29% lower risk of ILR recurrence than the 64Gy/32f schedule (adjusted HR=0.71 [95%CI: 0.52, 0.96]). Both schedules had similar toxicity profiles, with 55Gy/20f having slightly lower risk (RD=-3.37% [95%CI: -11.85%, 5.10%]) of grade 3/4 late bladder or rectum symptom than 64Gy/32f.

Interpretation

55Gy/20f is superior to 64Gy/32f for ILRC, and is non-inferior in terms of toxicity and OS. 55Gy/20f should be adopted as standard of care for bladder preservation in this patient population.

Funding: Cancer Research UK

Introduction

Bladder-preservation therapy is an alternative to surgery for the management of muscle invasive bladder cancer. Typically, this comprises pre-treatment staging with trans-urethral resection of tumour and cross-sectional imaging followed by radiotherapy with or without a radiosensitiser. It may also be preceded by neoadjuvant chemotherapy. Combining radiation with a radiosensitiser gives similar rates of disease-specific and overall survival rates (~50% at 5 years) when compared to surgery.(1-4) The two largest phase III randomised control trials of bladder preservation showed benefit using either chemotherapy (BC2001(3, 5)) or hypoxia modifying therapy (BCON(1)) with radiation.

Both trials permitted two commonly used radiotherapy fractionation schedules, 55 Gy in 20 fractions and 64 Gy in 32 fractions (f). Although there has been no direct comparison in the literature, published series suggest that outcome and late toxicity are comparable.(6) This work aimed to assess whether 55Gy/20f is non-inferior to 64Gy/32f in terms of invasive loco-regional control and late bladder and bowel toxicity using combined data from these two randomised phase III trials.

Methods

Study design and participants

BC2001 (NCT00024349) is a phase III randomised trial with a partial 2-by-2 factorial design.(3, 5) Between August 3, 2001 and April 28, 2008, 458 patients from 45 UK sites with a diagnosis of transitional cell bladder carcinoma, T2-T4, metastasis-free and suitable for radical radiotherapy, were recruited. Patients were randomised 1:1 to receive (1) radiotherapy with or without concomitant chemotherapy with fluorouracil (5-FU 500mg/m2 D1-5 and D16-20) and mitomycin C (MMC 12mg/m2 D1); and/or (2) standard whole-bladder radiotherapy or reduced high dose volume radiotherapy with tumour boost. Recruitment to both randomisations were optional depending on eligibility and patient preference.

BCON (NCT00033436) is a phase III randomised trial with parallel design.(1) Between November 15, 2000 and April 24, 2006, 333 patients from 13 UK sites with a diagnosis of transitional cell bladder carcinoma, T1G3-T4a and metastases-free were randomised to radiotherapy with or without hypoxia modification with carbogen (2% CO_2 and 98% O_2 at 15 l/min for 5 minutes prior to and during radiotherapy), and nicotinamide (orally at 40-60 mg/kg 1.5-2 hours before each fraction).

The two trials recruited patients from similar MIBC populations (appendix p1). In both trials, fractionation schedule (64Gy/32f or 55Gy/20f) was chosen by each participating centre according to local standard practice. Radiotherapy was delivered using a conventional or 3D-conformal technique

with an empty bladder. An expansion of 1.5 cm was used from clinical target volume to planned target volume. Pelvic lymph nodes were not included in the clinical target volume. Usually all patients from the same site were treated with the same fractionation schedule, but with 6 exceptions (4 BC2001, 2 BCON). Both studies followed the principles of Good Clinical Practice, and all participants provided written informed consent.

Initial staging was ascertained in both trials by cystoscopic examination and biopsy to confirm histological diagnosis, computed tomography (CT) or magnetic resonance (MR) of the abdomen and pelvis, and chest radiography (chest CT also allowed in BC2001).

Tumour control was assessed in BC2001 by means of physical examination, chest radiography, and rigid or flexible cystoscopy at six, nine, and twelve months after randomisation, and then annually. Biopsy of the tumour bed and normal bladder was mandated at six months and was repeated as indicated at subsequent cystoscopies. CT of the abdomen and pelvis was performed at one and two years after randomisation and then as indicated. In BCON, cystoscopic examination occurred six months after radiotherapy, and six-monthly for up to five years; CT and upper tract endoscopy were conducted when indicated. Management of patients following relapse was according to local practice in both trials. In BC2001, annual follow-up for disease events (recurrence of local/distant disease) and patient status was prospectively collected up to July 2016. In BCON, recruiting sites were contacted in 2018 to obtain long-term survival data (recurrence of local/distant disease, patient status), with a data lock in October 2018.

Both trials measured late toxicity up to five years post-radiotherapy using the Late Effects Normal Tissue Task Force/Subjective, Objective, Management, Analytic (LENT/SOMA)(7, 8) tool. In BCON, only urinary and rectal dysfunction sub-scales were recorded, and assessed more frequently (three-monthly year 1, six-monthly years 2-5) than BC2001 (three-monthly year 1, annually thereafter). In the BC2001 trial, health-related quality-of-life (HRQoL) was assessed at end of treatment, six and twelve months post-randomisation and then annually to five years using the Functional Assessment of Cancer Therapy-Bladder cancer module (FACT-BL).(9) A similar HRQoL schedule was planned in BCON, but data return was sparse and analysis not pursued.

Further details of the key features of both trials are provided in the appendix (p2).

Outcomes:

Based on information available in both trials, we defined common endpoints for this meta-analysis. The co-primary endpoints were invasive loco-regional control (ILRC) and late rectum and/or bladder toxicity.

ILRC was defined as the rate free of muscle-invasive bladder recurrence or recurrence in pelvic nodes (invasive loco-regional recurrence, ILRR). The time point of interest for the ILRC estimate was three years. To account for the difference in the length of disease follow-up assessments between trials, the window of observation was set at five years. Patients were therefore censored at five years if known to be alive and disease-free; or at last known disease assessment if alive and disease-free with <5 years follow-up; or at date of distant recurrence (unless a ILRR was diagnosed within 30 days following diagnosis of distant recurrence, to account for delay in confirming diagnoses); or at date of diagnosis of second primary (only collected in BC2001); or at death due to any cause (if recurrence-free).

Late toxicity was measured by the proportion of patients who experienced a grade three or greater (G3+) rectum or bladder adverse event as assessed by the LENT/SOMA scale, over five years from randomisation.

The secondary endpoint overall survival (OS) was defined as time from randomisation to death due to any cause. Patients alive at their last known follow-up time were censored. All follow-up available in either trial was used for this endpoint. A post-hoc exploratory analysis of bladder-cancer specific survival was conducted. Exploratory endpoints included change from baseline in HRQoL (BC2001 only).

Statistical analyses

Individual patient data (IPD) were combined into one dataset. Given the fractionation schedules were not randomised and confounding was likely, a one-stage IPD meta-analysis approach was chosen due to its flexibility to adjust for potential confounders(10-12) while ensuring that clustering within each trial was preserved.(13) There were differences in baseline data collection, which impacted on adjustment for confounders. Forest plots of fractionation effects for each outcome were used to explore the degree of overlap between the 95% confidence intervals (95%CI) of each trial.

The study hypothesised that 55Gy/20f was non-inferior to 64Gy/32f in terms of disease control and late toxicity. For each endpoint, non-inferiority would be declared if the upper limit of the 95% confidence interval of the estimated fractionation differences was smaller than the non-inferiority margin. The pre-specified non-inferiority margin for ILRC was a hazard ratio (HR) of HR_{NI}=1.25, and for late bladder and bowel toxicity, an absolute risk difference (RD) of RD_{NI}=10%.

All patients in the BCON and BC2001 trials who received at least one fraction of radiotherapy and for whom the fractionation schedule was known were included in the meta-analysis. Baseline imbalance was investigated using standardised differences,(14, 15) which provide a common scale (in %) for the magnitude of imbalance between fractionation groups for all baseline variables. Any variables with

>10% standardised difference were considered potential confounders and investigated in the meta-analysis.

For each time-to-event endpoint, a crude analysis to estimate the relative difference (HR) between fractionation schedules was first performed by fitting a stratified Cox proportional hazards model with fractionation schedule as the predictor, a frailty term for site clustering, and stratifying by trial. An adjusted HR for fractionation effect was fitted similarly, but incorporating trial(s) intervention (allocated use of concurrent radiosensitiser), pre-specified prognostic factors and any potential confounder due to baseline imbalance, or showing univariate association (at a significance level of 0.05) with the endpoint. Pre-specified prognostic factors for ILRC were age, sex, tumour stage, use of neoadjuvant chemotherapy and extent of resection; for OS were age and sex.(16) Model assumptions were assessed by graphical assessment of residuals. A likelihood ratio test for heterogeneity of fractionation effect across trials was performed by considering an extended model including the interaction of fractionation schedule and trial.

For the analysis of G3+ rectum/bladder toxicities within five years, toxicities reported ≤3 months prior to first recurrence or bladder cancer death were treated as missing to avoid interpreting recurrence symptoms as toxicities. The absolute risk difference (RD) between fractionation schedules in having G3+ rectum/bladder toxicity over five years was estimated using a generalised linear binomial model with random intercept for centre, to account for clustering within sites.(17) A crude model was first fitted with fractionation schedule and including trial as a fixed effect. In the adjusted analysis, we also included the trial(s) intervention, age, sex, and any confounders identified as imbalanced at baseline, or associated to the toxicity endpoint in univariate analyses. Heterogeneity between trials was explored considering an interaction effect between fractionation schedule and trial.

Pre-planned subgroup analyses included exploring the fractionation effect within trial and for patients who received radiotherapy alone; a 1% significance level was used in these analyses. The effect of fractionation schedule on HRQoL was explored in the BC2001 trial only, employing similar methods as used for the trial's HRQoL substudy.(18)

Data were analysed using Stata (version 15.0)(19) and the R(20) (version 3.6.0) survival and geepack(21) packages. Analysis was based on a data snapshot taken on July 11, 2016 for BC2001 and October 1, 2018 for BCON.

Expanded details of statistical methods used are provided in the appendix (pp 3-4).

Role of the funding source

The funders had no role in the study design, data collection, data analysis, data interpretation, or writing of the report. The corresponding author had full access to all data in the study and had final responsibility for the decision to submit for publication.

Results

In BC2001, fractionation schedule was unknown for 2 patients. Of the 456 included, 279 (54.6%) received 32f, while 177 (45.4%) received 20f. Median follow-up was 118 months (first quartile Q1 to third quartile Q3: 100 to 137). In BCON, fractionation schedule was unknown for five participants, and two further patients found to have metastases at baseline were also excluded from the meta-analysis. Of the 326 included, 97 (30%) received 32f and 229 (70%) underwent 20f. Median follow-up was 159 months (Q1-Q3 91 to 181). The combined dataset therefore consisted of 782 patients recruited from 50 sites (eight sites common to both trials), 376 (48%) who received 32f, 406 (52%) who received 20f (Figure 1). Median follow-up in the combined dataset was 120 months (Q1-Q3 99 to 159).

Patient characteristics in the combined dataset show imbalances between fractionation groups (Table 1) with respect to stage (standardised difference, StDiff=42.6%), grade (StDiff=11.1%) and extent of resection (StDiff=36.4%). The 20f group included more patients with T3 or higher disease (112/406, 27.6%) than the 32f group (43/376, 11.4%). In the 20f there were also more patients who had incomplete resection (143/406, 36.5%) than the 32f group (103/376, 27.6%). In BC2001, 75 patients (26.9%) of 279 in the 32f group received reduced high dose volume radiotherapy as part of the radiotherapy comparison, while 35 (19.8%) of 177 did so in the 20f cohort. All patients in BCON received standard whole bladder radiotherapy.

218 (27.9%) patients of 782 experienced an ILRR within five years, 106 events (28.2%) in 376 patients in the 32f group and 112 events (27.6%) in 406 patients in the 20f group. Median follow-up for ILRC analysis was 60 months (Q1-Q3 21 to 60). Observed Kaplan-Meier ILRC rates over time per trial and fractionation groups are summarised in Figure 2A. In the combined dataset, the crude one-stage meta-analysis showed that patients receiving 20f schedule had an estimated 17% lower hazard of ILRR than patients who received the 32f schedule (HR=0.83 [95%CI: 0.63, 1.10]). After accounting for trial, age, sex, trial(s) intervention, extent of resection, tumour stage, haemoglobin and use of neoadjuvant chemotherapy, the effect increased to a 29% reduction in hazard (HR=0.71 [95%CI: 0.52, 0.96]) (represented in Figure 3A, and extended details of the modelling provided in appendix p5). As the upper limit of the 95%CI for both the crude and adjusted estimates is lower than the pre-specified HR_{NI} (1.25), non-inferiority of the 20f schedule could be concluded. Moreover, since the 95%CI for the adjusted HR estimate excludes HR=1 (no effect), the adjusted analysis indicates that the 20f schedule

significantly improves ILRC rate at the 5% level. The benefit of 20f in ILRC was also seen in patients receiving radiotherapy alone (HR=0.72 [95%CI: 0.49, 1.05]), and in patients receiving radiotherapy with concurrent radiosensitiser (HR=0.68, [95%CI: 0.42, 1.11]). No significant heterogeneity across sites or trials was found.

571 (73%) of 872 patients died while on follow-up, 273 (72.6%) of 376 in the 64Gy group and 298 (73.4%) of 406 in the 55Gy group. Observed Kaplan-Meier OS rates over time per trial and fractionation groups are summarised in Figure 2B. The crude one-stage meta-analysis showed that patients who received 20f have 1% lower hazard of death than patients who received 32f (HR=0.99 [95%CI: 0.78, 1.28]). After accounting for age, sex, trial(s) intervention, extent of resection, tumour stage and haemoglobin, the effect increased to 13% reduction (HR=0.87 [95%CI: 0.72, 1.06]) (Figure 3A extended details in appendix p6). If the same HR_{NI}=1.25 was considered for OS, only the adjusted model would suggest non-inferiority of the 20f schedule. Consistent fractionation estimates were observed for patients receiving radiotherapy alone (HR=0.92 [95%CI: 0.72, 1.18]) or with a concurrent radiosensitiser (HR=0.83 [95%CI: 0.62, 1.11]). No significant heterogeneity across sites or trials was found. No differences between fractionation groups were found in the exploratory analysis of bladder cancer-specific survival (adjusted HR=0.83 [95%CI: 0.66-1.05], appendix pp7).

Bladder and rectum LENT/SOMA toxicity data in the BC2001 trial were available for analysis in 203 patients (72.7%) of 279 receiving 32f and 120 patients (67.8%) of 177 who received 20f. In the BCON trial, bladder and rectum toxicity data were available in 75 patients (77.3%) of 97 in the 32f cohort and 175 patients (76.4%) of 229 in the 20f cohort. In the combined dataset, 278 patients (73.9%) of 376 who received 32f, and 295 patients (72.7%) of 406 who received 20f, had toxicity data available. Appendix p8 shows the distribution of baseline variables over fractionation groups in patients with data available for toxicity analysis.

The proportion of patients experiencing G3+ rectum or bladder toxicity within 5 years was similar in both fractionation groups, with 89/278 patients (32.0%) in the 32f cohort compared to 97/295 patients (32.9%) in the 20f cohort (Table 2). In the combined one-stage IPD meta-analysis, the crude analysis suggested a 2.88% lower risk of G3+ toxicity for patients receiving 20f versus 32f (risk difference, RD=-2.88% [95%CI: -11.15%, +5.39%]). Similar results were obtained after adjusting by age, sex, trial(s) treatment, and trial (RD=-3.37% [95%CI: -11.85%,+5.10%]) (Figure 3B, extended details in appendix p9). A similar difference in risk was found in a sensitivity analysis conducted without censoring toxicities within 3 months of a recurrence event (adjusted RD=-3.82% [95%CI: -11.88%, 4.24%]). As the upper limit of both the crude and adjusted 95%CI is smaller than RD_{NI}=10%, non-inferiority of 20f compared to 32f in five-year bladder and bowel toxicity can be concluded. In the

subgroup of patients who received radiotherapy only, the 20f group also had lower risks of G3+ toxicity (RD=-12.51 [95%CI: -23.84, -1.19]) than the 32f group, but in the subgroup of patients who received radiotherapy and a radiosensitiser, the 20f group presented higher risk of G3+ toxicity (RD=+7.32 [95%CI: -5.03, +19.67]). The test for interaction between trial intervention and fractionation group was significant (p=0.001).

In BC2001, baseline FACT-BL scores were balanced between fractionation schedules (appendix p10). Although there was a detrimental effect of the 20f at the end of treatment for the TOTAL score (estimated adjusted mean difference between fractionation groups -9.34 [99%CI: -18.36, -0.32], p=0.008), this difference was no longer statistically significant at one year (-1.29 [99%CI: -12.31, 9.72], p =0.76), nor at later times (appendix p11).

The combined models for ILRC, OS and late toxicity (appendix pp5-6, p9) also provided estimates for the radiosensitiser effect. Significantly improved HRs were seen with a radiosensitiser compared with radiotherapy alone for ILRC (HR=0.65 [95%CI: 0.49, 0.87]) and OS (HR=0.83 [95%CI: 0.70, 0.98]); there was no significant increase for late G3+ rectum or bladder toxicity (RD=-1.40 [95%CI: -9.43,+6.63]). These results confirm the benefit of the interventions in both trials.

Discussion

To our knowledge, this is the first study comparing the outcomes of conventional fractionation with moderately hypofractioned radiotherapy for MIBC. Hypofractionated radiotherapy with 55Gy/20f is non-inferior to conventional dose and fractionation with 64Gy/32f. The results indicate superior ILRC with hypofractionated radiotherapy despite the patients treated with 55Gy/20f having poorer prognostic factors. This finding was confirmed across all subgroups regardless of the intervention with moderately hypofractionated radiotherapy being advantageous whether a patient was treated with radiotherapy alone or radiotherapy with radiosensitisation. As such there is a cogent argument for 55Gy/20 fractions being adopted as the standard of care in this patient group. Many studies from outside the UK advocate tri-modality treatment with a complete trans-urethral resection of bladder (TURBT) being essential to undertake bladder preservation.(22-24) Both BC2001 and BCON had a high rate of local control despite high proportions of patients with incomplete resections. Though undertaking a complete TURBT may be optimal, the results of both trials suggest that bladder preservation can be achieved even in its absence.

Bladder cancer is considered a rapidly proliferating cancer with an α/β of 10Gy(25) and there is evidence to suggest a loss (γ) of 0.2-0.36 Gy per day after approximately 5 weeks of treatment due to

repopulation (appendix p12).(26) Using α/β of 10 Gy without accounting for overall time suggests that 66Gy/32f and 55Gy/20f have Biologically Effective Dose (BED) of 76.8Gy and 70.1Gy respectively. This difference was reduced when a time factor was included, with the maximum reduction for kick-off time (Tk) of 28 days or less. If BED was calculated with γ=0.36 and Tk=28 days, the 64Gy/32f and 55f/20f have BED of 71Gy and 70.1Gy respectively. For both schedules to be equivalent without a time factor, an α/β of 2 for bladder cancer would be required. From the data, repopulation is significant in the longer 32-fraction regimen. Investigators previously reported repopulation after 5 weeks.(26, 27) Our study suggests that repopulation occurs from as early as four weeks. Further data for different radiotherapy schedules are required for model optimisation, but the reality is likely to be a combination of an α/β lower than 10 with a significant effect of repopulation. The overall treatment time for rapidly proliferating cancers at high risk of repopulation is critical, with evidence of detrimental outcomes when treatment is prolonged. Guidelines are available for accommodating unexpected gaps in treatment.(28)

Enhanced acute toxicity is a concern with a shortened schedule(29) but unfortunately differences in data collection between the trials limit our assessment of acute toxicity. HRQoL from BC2001 did suggest worse quality of life at the end of treatment for the hypofractionated schedule, but this did not result in excess treatment interruptions; and after 6 months no difference in HRQoL was seen.

While concern is often expressed about the risk of late toxicity with hypofractionated radiotherapy, this meta-analysis showed no significant difference in late toxicity between fractionation regimens. Despite this, care should be used when extrapolating this data to radiosensitisation with other treatments such as immunotherapy where there may be a greater impact of hypofractionation.(30) Furthermore, there was no difference in patient-reported HRQoL after recovering from acute toxicity in the BC2001 trial. The published 5-year patient reported outcomes show excellent preservation of function with both fractionation schedules throughout the follow-up period.(18) The subgroup analysis for toxicity indicating a detrimental effect of 55Gy/20f in patients receiving a concurrent radiosensitiser should be interpreted with caution, as any differences might relate to the combined benefit from the sensitiser and hypo-fractionation prolonging the recurrence-free time, resulting in these patients having longer follow-up to collect toxicity data.

There are innate challenges when combining data from two phase III randomised control trials with no pre-planned meta-analysis. Acknowledging the limitations in this study, the primary outcome here differs from the primary endpoints in BC2001 (loco-regional control, including non-muscle invasive bladder recurrences) and BCON (local relapse-free survival, including invasive recurrences and death). Since the BCON dataset contains information on recurrence of muscle invasive lesions only, the

BC2001 secondary endpoint ILRC was chosen as the primary endpoint for this meta-analysis as it could be defined in both trials. Moreover, there were differences between trials in data collection with toxicity reported more frequently in BCON than in BC2001. This was overcome using cumulative reporting of adverse events over a common reporting period. BC2001 included prospective annual long-term follow-up beyond five years to collect basic information on the events of interest, while in BCON a one-off retrospective data collection was conducted to update follow-up. To overcome this, ILRC was analysed only within five years of follow-up. Finally, this is not a randomised comparison but is driven by institutional practice differences, as reflected in the differing proportional split in fractionation between the two trials: 45% BC2001 patients received hypofractionated radiotherapy as compared to 70% in BCON. Although case-mix differences were included in the modelling, there may be unanticipated effects. A prospective randomised clinical trial comparing both schedules would ideally provide the definitive evidence to a question of optimal radiotherapy schedule, but it is unlikely to be feasible given the logistics, numbers of patients and length of follow up required. In the absence of such evidence, an individual patient data meta-analysis using data from the two largest randomised control trials for bladder preservation is the best approach.

There are numerous socio-economic advantages to shorter treatment protocols in any healthcare system. Where there is evidence of superiority of treatment with no difference in long-term side-effects or detriment to the patient experience, the protocol should be adopted as standard-of-care. Therefore we recommend 55Gy/20f should be adopted as standard-of-care for bladder preservation in this patient population.

Table 1 – Summary of baseline characteristics in BC2001, BCON and the combined dataset

Variable		BC2001			BCON			COMBINED DATASET		
		64Gy/32f (n=279)	55Gy/20f (n=177)	Std diff (%)	64Gy/32f (n=97)	55Gy/20f (n=229)	Std diff (%)	64Gy/32f (n=376)	55Gy/20f (n=406)	Std diff (%)
Sex	Male	229 (82.1)	140 (79.1)	7.5	80 (82.5)	181 (79.0)	8.7	309 (82.2)	321 (79.1)	7.9
Age (years)	Mean (SD)	71.4 (8.7)	71.6 (7.9)	1.3	72.6 (7.6)	73.0 (7.8)	-5.4	71.7 (8.4)	72.4 (7.8)	7.9
Trial(s) intervention	Radiotherapy + radiosensitiser	111 (39.8)	71 (40.1)	0.7	49 (50.5)	116 (50.7)	0.3	160 (42.6)	187 (46.1)	7.1
Radiotherapy treatment	Reduced High Dose Volume RT	75 (26.9)	35 (19.8)	16.9	0 (0.0)	0 (0.0)	--	75 (19.9)	35 (8.6)	32.8
Tumour stage	1	1† (0.4)	0 (0.0)	47.6	13 (13.4)	17 (7.5)	32.1	14 (3.7)	17 (4.2)	42.6
	2	251 (90.0)	129 (72.9)		68 (70.1)	147 (64.5)		319 (84.8)	276 (68.2)	
	3	20 (7.2)	40 (22.6)		14 (14.4)	54 (23.7)		34 (9.0)	94 (23.2)	
	4	7 (2.5)	8 (4.5)		2 (2.1)	10 (4.4)		9 (2.4)	18 (4.4)	
	Unknown	0	0		0	1		0	1	
Tumour grade	1	1 (0.4)	0 (0.0)	13.9	0 (0)	0 (0)	9.4	1 (0.3)	0 (0.0)	11.1
	2	40 (14.4)	19 (10.9)		16 (16.5)	30 (13.2)		56 (15.0)	49 (12.2)	
	3	236 (85.2)	156 (89.1)		81 (83.5)	198 (86.8)		317 (84.8)	354 (87.8)	
	Unknown	2	2		0	1		2	3	
Extent of resection	Biopsy/ Not resected	24 (8.7)	23 (13.2)	34.4	25 (25.8)	62 (28.4)	19.6	49 (13.1)	85 (21.7)	36.1
	Complete	175 (63.4)	81 (46.6)		46 (47.4)	83 (38.1)		221 (59.3)	164 (41.8)	
	Partial	77 (27.9)	70 (40.2)		26 (26.8)	73 (33.5)		103 (27.6)	143 (36.5)	
	Unknown	3	3		0	11		3	14	
Neoadjuvant chemo	Yes	65 (23.3)	69 (39.0)	34.4	0 (0.0)	0 (0.0)	--	65 (17.3)	69 (17.0)	0.8
Haemoglobin (g/dl)	Mean (SD)	13.1 (1.8)	12.6 (1.8)	27.4	13.8 (1.7)	13.6 (1.6)	11.3	13.2 (1.8)	13.1 (1.8)	5.7
	Unknown	1	0		3	2		4	2	

SD: standard deviation; Std diff: Standardised difference - difference in means or proportions divided by its standard error; it is therefore a measure of the average difference between groups expressed in standard deviation units. So std diff>+/-10% expresses that the observed difference between fractionation groups is more than 10% of the observed variability.

Percentages calculated over total number of patients with non-missing values

†This tumour was deemed to be pathological stage T1, but radiologic staging confirmed the tumour as T3. Therefore, the patient was considered to be eligible for the trial.

Table 2 - LENT/SOMA bladder or rectum toxicity up to 2 or 5 years after end of treatment across fractionation groups

	64Gy/32f (N=278)	**55Gy/20f (N=295)**
2-year late toxicity		
Rectum	7 (2.5%)	17 (5.8%)
Bladder	66 (23.7%)	74 (25.1%)
Rectum or bladder	69 (24.8%)	82 (27.8)
5-year late toxicity		
Rectum	8 (2.9%)	21 (7.1%)
Bladder	86 (30.9%)	88 (29.8%)
Rectum or bladder	89 (32.0%)	97 (32.9%)

Figure 1 – Trials profile

RT: Radiotherapy, MMC: mytomicin C; 5-FU: fluorouracil; RDHVRT: reduced high dose volume radiotherapy; CON: carbogen and nicotinamide; cRT: chemoradiotherapy. Gy: Gray, f: fractions.

Figure 2. (A) Observed invasive loco-regional control Kaplan-Meier estimate by trial and fractionation group (B) observed overall survival Kaplan-Meier estimate by trial and fractionation group

ILRC: Invasive Loco-Regional Control Rate; 3-year estimates presented with 95% confidence interval (95%CI). OS: Overall Survival, 5-year estimates presented with 95% confidence interval (95%CI).
*Number at risk – number of patients at risk of the event of interest at each timepoint. Number censored – indicates cumulative number of censored observations occurring up to the corresponding interval – e.g. in the BC2001 64Gy group, 41 patients censored between 0 and 1 year, 71 patients censored between 0 and 2 years, 88 patients censored between 0 and 3 years, etc.

Figure 3. (A) Forest plot of the fractionation effect 64Gy/32f vs 55Gy/20f for Invasive Loco-regional Control and Overall Survival (differences expressed in hazard ratios); (B) Forest plot of the fractionation effect 64Gy/32f vs 55Gy/20f for toxicity (differences expressed in absolute risk difference)

NI: non-inferiority. Hazard ratios represented in log-scale.
Invasive Loco-Regional Control combined estimates adjusted for age, sex, randomised treatment, extent of resection, tumour stage, haemoglobin and neoadjuvant chemotherapy; model stratified by trial and random effect for centre.
Overall Survival combined estimates adjusted for age, sex, randomised treatment extent of resection, tumour stage, haemoglobin; model stratified by trial and random effect for centre.
Late rectum and/or bladder toxicity combined estimates adjusted for age, sex, randomised treatment, and trial; random intercept for centre.

Acknowledgements

This paper represents independent research supported by Cancer Research UK (C2094/A11365, C1491/A25351) and Experimental Cancer Medicine Centre funding (C1467/A7286). It is also supported by the National Institute for Health Research (NIHR) Biomedical Research Centre at The Royal Marsden NHS Foundation Trust and the Institute of Cancer Research, London, and by the NIHR Manchester Biomedical Research Centre, Manchester. The views expressed are those of the author(s) and not necessarily those of the NIHR or the Department of Health and Social Care.

BC2001 was supported by Cancer Research UK (CRUK/01/004) with programme grants to support the work of the CR UK Cancer Trials Unit, Birmingham (C547/A2606; C547/A6845; C9764/A9904) and ICR-CTSU (C1491/A9895; C1491/A15955; C1491/A25351). BCON was supported by Cancer Research UK (C2422/A2564); and the Mount Vernon Marie Curie Research Wing Translational Research Fund. Trials' recruitment was facilitated at participating sites by the National Institute for Health Research (NIHR)-funded National Cancer Research Network.

Grateful thanks to all the patients who participated in the BC2001 and BCON trials,; all involved staff at participating centres; and trials unit staff at ICR-CTSU, Birmingham CRUK-CTU and Mount Vernon Cancer Centre, including Hannah Gribble at ICR-CTSU who supported manuscript preparation and submission.

Author contributions

AC, NP, EH, NJ, RH and PH designed the trial.

NP, EH, RL, YS and PH managed the trial and trial data.

AC, NP, EH, RL, NJ, RH and PH developed the protocol.

NP, EH, RL, AC, YS and PH collected data.

NP, RO and EH did the statistical analyses.

AC, NP, EH, YS, RO, RM, CW, RL, SH, NJ, RH and PH interpreted the data.

AC, NP, EH, RM, CW, NJ, RH and PH wrote the manuscript.

NJ and RH were chief investigators of BC2001.

PH was chief investigator of BCON.

All authors reviewed the manuscript prior to submission.

Declaration of interest

AC reports grants from National Institute of Health Research, Manchester Biomedical Research Centre, grants from Cancer Research, UK, grants from Medical Research Council, UK, grants from Prostate Cancer, UK, grants from Bayer, UK, personal fees from Janssen Pharmaceutical, non-financial support from ASCO, grants and non-financial support from Elekta AB, outside the submitted work; .

NP has nothing to disclose.

EH reports grants from Cancer Research UK, during the conduct of the study; grants from Accuray Inc., grants from Varian Medical Systems Inc., outside the submitted work; .

YPS reports other from Elekta, other from Bayer, outside the submitted work.

RO has nothing to disclose.

RM has nothing to disclose

CW has nothing to disclose.

RL has nothing to disclose.

SH has nothing to disclose.

NJ has nothing to disclose.

RH reports grants, personal fees and non-financial support from Roche, grants and personal fees from Merck, Sharp, Gohme, grants from Elekta, personal fees from Jansen, personal fees from Nektar Pharmaceuticals, personal fees from Astellas, grants and non-financial support from NIHR Biomedical Research Centre Institute of Cancer Research and Royal Marsden FT, outside the submitted work.

PH reports grants from CANCER RESEARCH UK, during the conduct of the study.

Data sharing statement

The authors support the wider dissemination of information from the research we have conducted, and increased cooperation between investigators. Trial data is collected, managed, stored, shared and archived according to the relevant CTU Standard Operating Procedures in order to ensure the enduring quality, integrity and utility of the data. Formal requests for data sharing are considered in line with the relevant CTU procedures with due regard given to funder and sponsor guidelines. Requests are done in writing describing the nature of the proposed research and extent of data requirements. Data recipients are required to enter a formal data sharing agreement which describes the conditions for release and requirements for data transfer, storage, archiving, publication and Intellectual Property. Requests will be reviewed by the authors and as appropriate by the Trial Management Groups (TMG) in terms of scientific merit and ethical considerations including patient consent. Data sharing is undertaken if proposed projects have a sound scientific or patient benefit rationale as agreed by the authors and TMGs, as appropriate.

De-identified individual participant data, together with a data dictionary defining each field in the set, will be made available following approval of the request, as well as supporting documentation as required. Restrictions relating to patient confidentiality and consent will be limited by aggregating and anonymizing identifiable patient data. Additionally all indirect identifiers that may lead to deductive disclosures will be removed in line with Cancer Research UK Data Sharing Guidelines.

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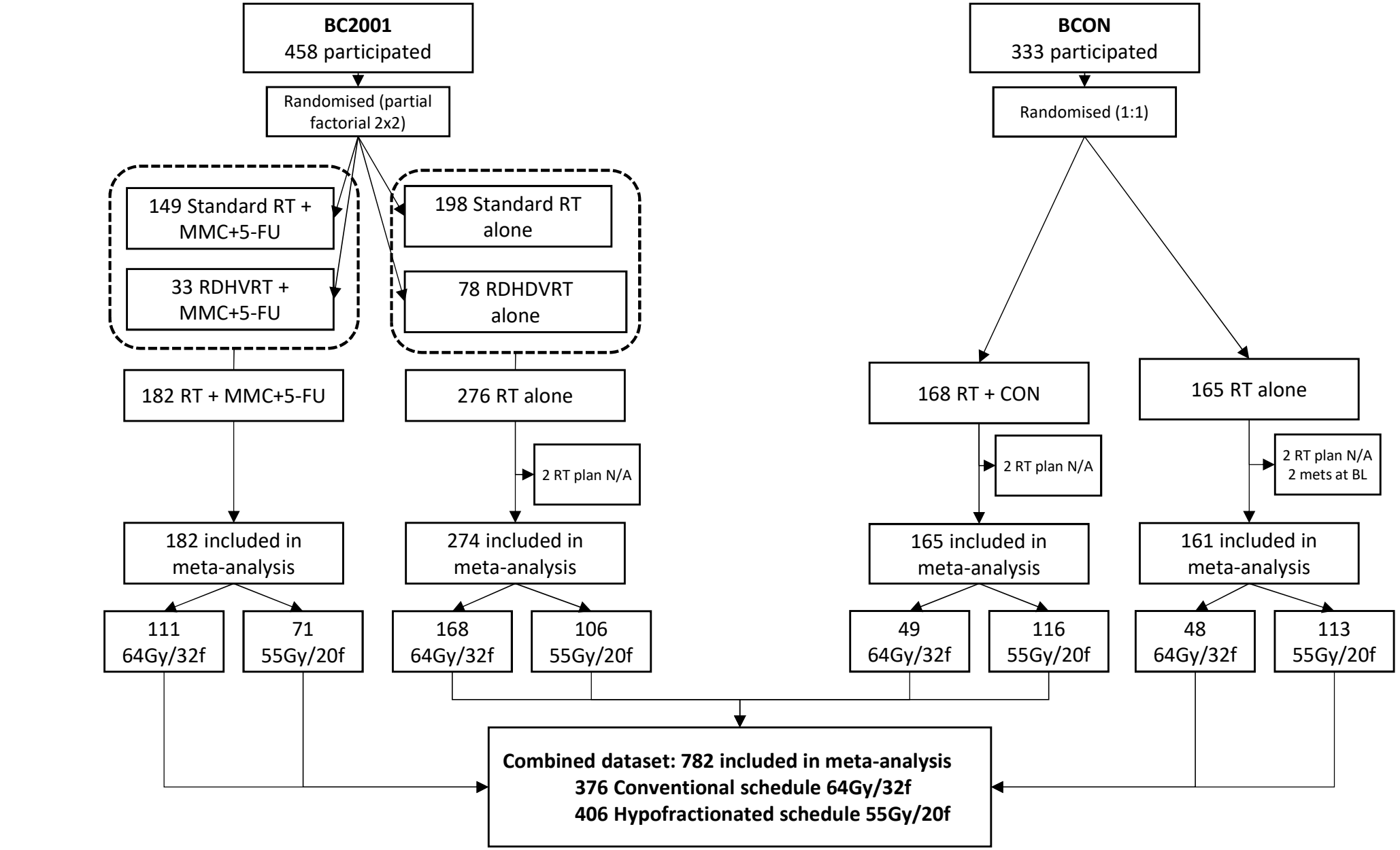


BC2001 CT randomisation (cRT vs RT)

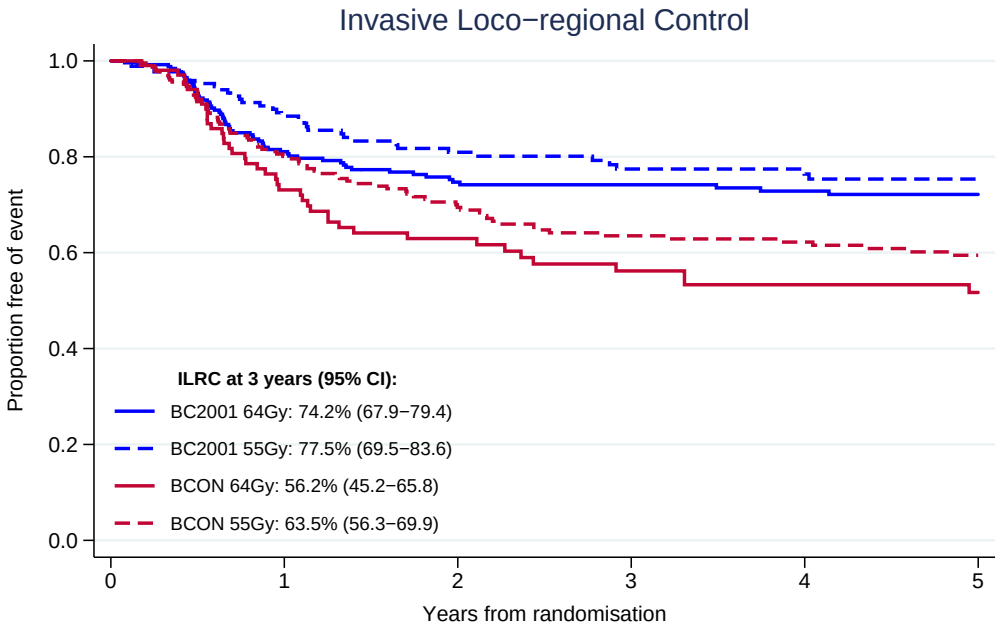
Excluded (n=98):

- 53 Were ineligible for chemotherapy
- 34 Withdrew or were withdrawn by physician
- 4 Had other reasons
- 7 Had unknown reasons

BC2001 RT randomisation (sRT vs RHDVRT)

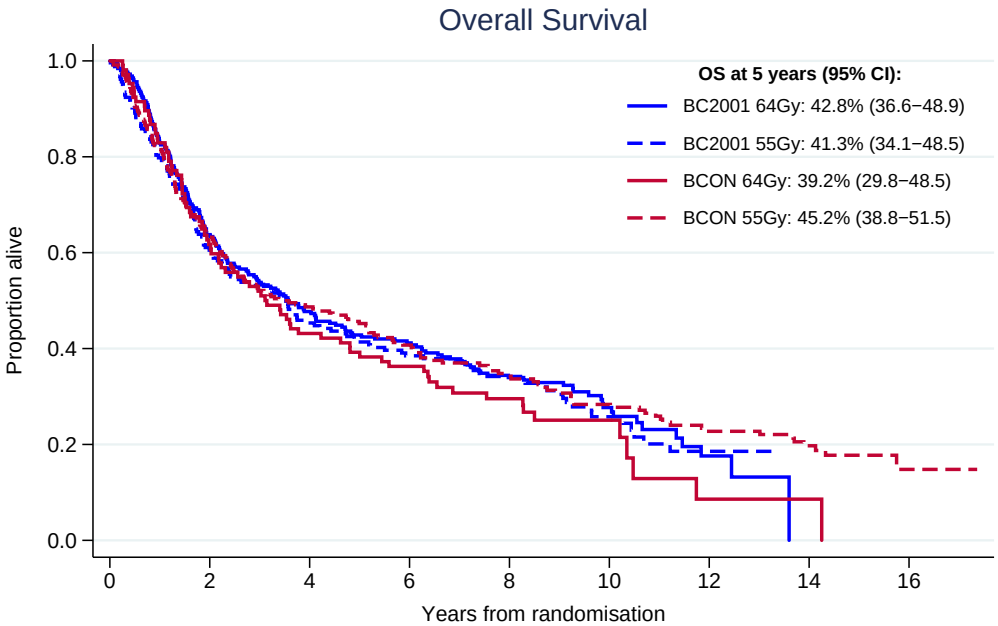
Excluded (n=239):

- 84 entered BC2001 after RT randomisation closed
- 54 centre not participating
- 47 multiple tumours
- 44 physician/patient decision
- 10 Admin/unknown reasons



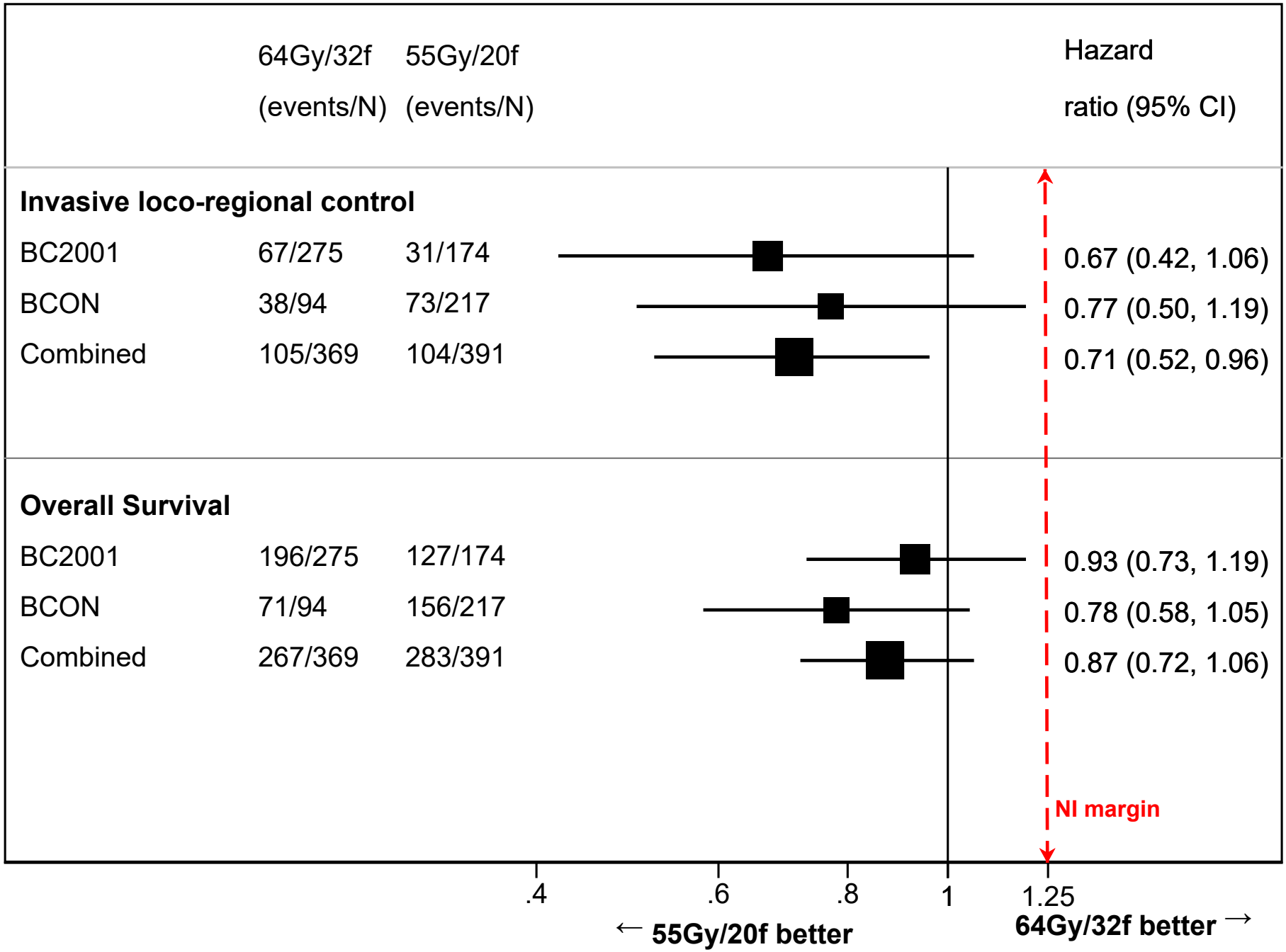
Number at risk (censored)*

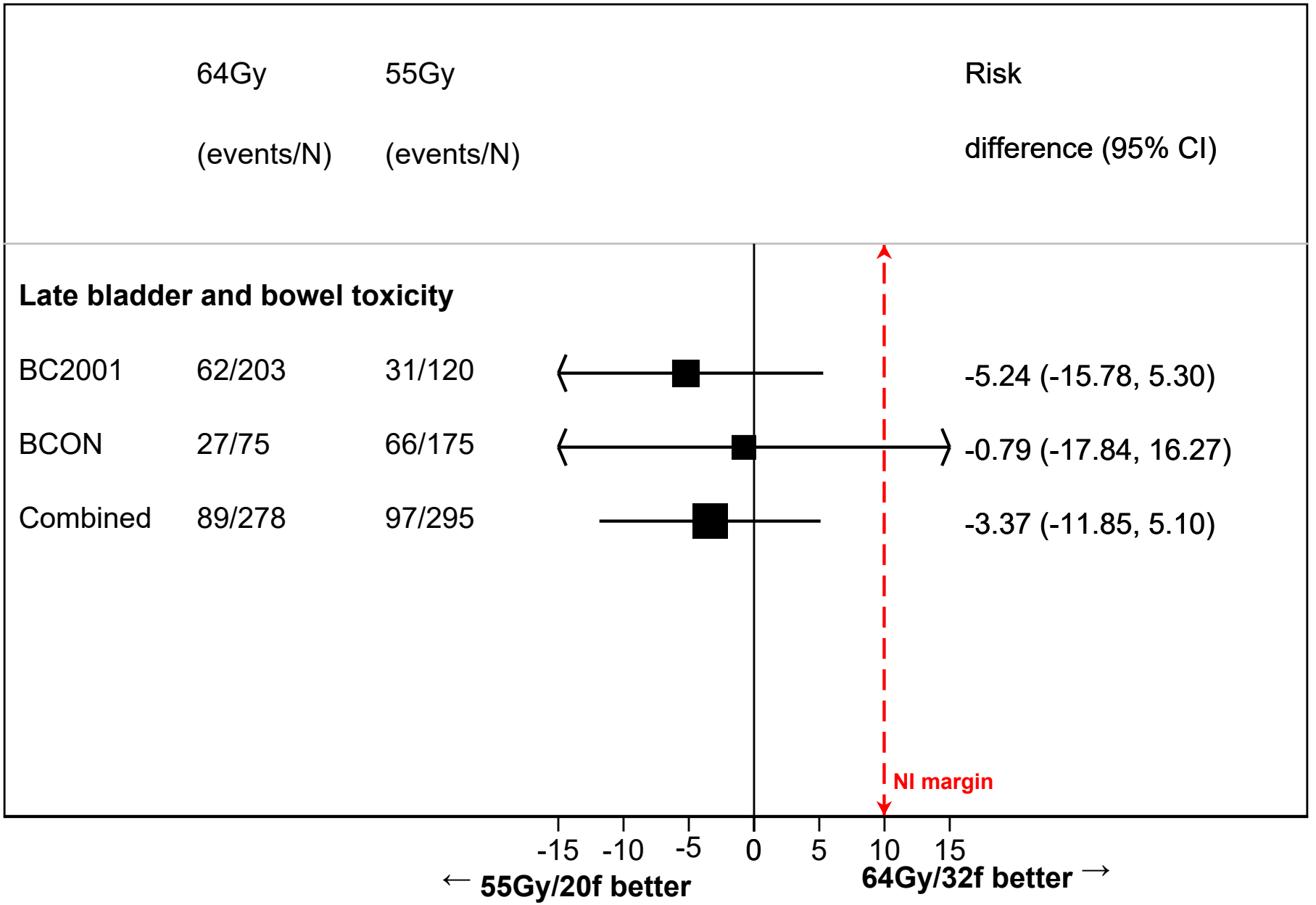
BC2001 64Gy	279	(41)	188	(71)	145	(88)	127	(100)	113	(110)	102	(.)
BC2001 55Gy	177	(42)	117	(56)	93	(65)	80	(75)	69	(78)	65	(.)
BCON 64Gy	97	(9)	66	(16)	50	(22)	39	(24)	35	(26)	32	(.)
BCON 55Gy	229	(30)	157	(45)	122	(58)	99	(63)	92	(70)	81	(.)



Number at risk (censored)*

BC2001 64Gy	279	(4)	173	(7)	126	(9)	107	(18)	80	(57)	32	(73)	8	(73)				
BC2001 55Gy	177	(2)	105	(5)	76	(7)	62	(13)	49	(30)	22	(40)	7	(40)				
BCON 64Gy	97	(1)	61	(2)	43	(4)	34	(10)	22	(22)	7	(23)	2	(24)	1	(24)	0	(24)
BCON 55Gy	229	(3)	143	(4)	109	(23)	74	(28)	58	(30)	46	(32)	36	(42)	22	(58)	3	(58)


64Gy/32f (events/N)
55Gy/20f (events/N)
Hazard ratio (95% CI)
Invasive loco-regional control
BC2001 67/275 31/174 0.67 (0.42, 1.06)
BCON 38/94 73/217 0.77 (0.50, 1.19)
Combined 105/369 104/391 0.71 (0.52, 0.96)
Overall Survival
BC2001 196/275 127/174 0.93 (0.73, 1.19)
BCON 71/94 156/217 0.78 (0.58, 1.05)
Combined 267/369 283/391 0.87 (0.72, 1.06)
NI margin
.4
.6
.8
1
1.25
← 55Gy/20f better
64Gy/32f better →


64Gy
(events/N)
55Gy
(events/N)
Risk
difference (95% CI)
Late bladder and bowel toxicity
BC2001
62/203
31/120
-5.24 (-15.78, 5.30)
BCON
27/75
66/175
-0.79 (-17.84, 16.27)
Combined
89/278
97/295
-3.37 (-11.85, 5.10)
NI margin
-15
-10
-5
0
5
10
15
← 55Gy/20f better
64Gy/32f better →

Hypofractionated radiotherapy in muscle-invasive bladder cancer: an individual patient data meta-analysis of the BC2001 and BCON trials

Supplementary material

1 Inclusion and exclusion criteria of the BC2001 and BCON trials

BC2001	BCON
Inclusion criteria: • Aged 18 or over • Histologically proven invasive bladder carcinoma (adenocarcinoma, transitional or squamous cell carcinoma) • Localised muscle invasive carcinoma either surgically or by imaging (T2-T4a N0 M0) • Patients with multiple tumours at the time of randomisation were not eligible for the radiotherapy volume randomisation but could be randomised to whole bladder radiotherapy with or without synchronous chemotherapy • WHO performance status of grade 0 to 2 • Leucocytes > 4.0x10P9P/L, platelets > 100x10P9P/L • GFR > 25ml/min • Serum bilirubin < 1.5 upper limit of reference range (ULRR) ALT or AST < 1.5 x ULRR • Patient available for long term follow up, and in the opinion of investigator, able to receive a radical course of radiotherapy • Patient's written informed consent	**Inclusion criteria:** • Age over 18 years • Histologically proven transitional cell carcinoma of the bladder • Muscle invasive carcinoma (Stage T2 or T3) of any grade, high grade (G3) superficial bladder carcinoma (T1) or prostatic invasion (T4a) • Ability to give informed consent • Capable of complying with the use of a closed breathing system delivering carbogen through either a mask or a mouthpiece with nasal clip • Any WHO performance status
Exclusion criteria: Patients with any of the following were not eligible for the trial: • Uncontrolled systemic disease which would preclude the patient from the study • Pregnancy • Other malignancy within the previous 2 years (other than adequately treated BCC of the skin or adequately treated in situ carcinoma of the cervix uteri) • Previous malignancy that is likely to interfere with protocol treatment • Inflammatory bowel disease • Previous pelvic radiotherapy • Bilateral hip replacements compromising accurate radiotherapy planning	**Exclusion criteria:** • Squamous or adenocarcinoma of the bladder • Locally advanced T4b carcinoma • The presence of distant metastasis or enlarged pelvic lymph nodes on CT staging scan of the pelvis • Co-existing respiratory disease with reduced respiratory drive which would make a delivery of 95% oxygen contra- indicated • Impaired renal or hepatic function resulting in serum creatinine or bilirubin more than twice the normal range • Ischaemic heart disease or peripheral vascular disease requiring treatment with ACE inhibitors

2 Summary of key features (radiotherapy treatment, baseline and follow-up assessments) in the BC2001 and BCON trials

	BC2001 Standard arm	BC2001 Reduced High dose volume arm	BCON
Staging investigations	Cystoscopy, Biopsy+/- TURBT CT/MR abdomen pelvis Chest XR1	Cystoscopy, Biopsy+/- TURBT CT/MR abdomen pelvis Chest XR1	Cystoscopy, Biopsy+/- TURBT CT/MR abdomen pelvis Chest XR
Clinical target volume (CTV)	Planned with empty bladder Bladder plus extravesical bladder tumour	Planned with empty bladder CTV1 Bladder plus extravesical bladder tumour CTV2 Gross tumour volume	Planned with empty bladder Bladder plus extravesical bladder tumour
Lymph node radiotherapy	No	No	No
CTV to planning target volume (PTV)	1.5cm	1.5cm	1.5cm
Radiotherapy technique	Conventional or conformal 3 fields	Conformal 2 phase or concomitant boost 3 fields	3d Conformal 3 or 4 fields
Dose	64Gy in 32fractions (f) over 6.5 weeks or 55Gy in 20fractions over 4 weeks	64Gy in 32f or 55Gy in 20f to PTV2 80% of dose to PTV1 outside PTV2	64Gy in 32fractions over 6.5 weeks or 55Gy in 20fractions over 4 weeks
Health-Related Quality of life (HRQoL)	Yes	Yes	No[2]
Follow up cystoscopy	6 and 9 months post randomisation then annually	6 and 9 months post randomisation then annually	6 months post radiotherapy treatment then 6 monthly to 5 years
Follow up imaging	Chest X-Ray 6, 9, 12 months post randomisation then annually CT abdomen/pelvis year 1 and 2 and as clinically indicated	Chest X-Ray 6, 9, 12 months post randomisation then annually CT abdomen/pelvis year 1 and 2 and as clinically indicated	As clinically indicated

TURBT: transurethral resection of bladder tumour; CT: computer tomography; MR: magnetic resonance; XR: X-rays
1 Chest CT also allowed
2 HRQoL planned in BCON, but data return was sparse and analysis not pursued.

3 Statistical Methods: expanded details

Individual patient data (IPD) were obtained from both trials and combined into one dataset. A study identifier unique to each trial was created. Variables available in both datasets were recoded to common names and definitions. Given that the comparison between fractionation schedules was not randomised, and therefore confounding was likely to be present, a one-stage IPD meta-analysis approach was chosen, which was more flexible to adjust for potential confounders.[1-3] In a one-stage approach, analysis was based on the combined dataset, ensuring that clustering within each trial was preserved.[4] There were differences in baseline data collection, which impacted on adjustment of the confounders in the meta-analysis. Forest plots of fractionation effects for each outcome were used to explore the degree of overlap between the 95% confidence intervals (95%CI) of each trial.

The hypothesis of the study was that the hypofractionated RT schedule 55Gy/20f was non-inferior to 64Gy/32f, both in terms of disease control rate and late toxicity. For each endpoint, non-inferiority would be declared if the upper limit of the 95% confidence interval of the estimated fractionation differences was smaller than the non-inferiority margin.

Crude power calculations of non-inferiority based on the number of patients recruited into each trial were performed. Power was computed assuming there were truly no differences between fractionation schedules. As the meta-analysis involved adjusted estimates of fractionation differences, the power was expected to be higher than the below crude estimates. For the primary endpoint ILRC, with a sample size of 791 patients in the combined dataset, we would have 61% power to conclude that 55Gy/20f is non-inferior to 64Gy/32f, assuming an unadjusted log-rank comparison of the fractionation groups, one-sided 0.025 alpha, similar size of fractionation groups (1:1 ratio), and a non-inferiority margin set at hazard ratio of 1.25. If the 2 year-survival in the 64Gy group was 75% (as in BC2001), this margin corresponds to a 2-year rate in the 55Gy being no worse than 69%. For late toxicity, assuming the proportion of GI/GU grade 3 or more LENT/SOMA toxicity overall was 40% in the 64Gy group (from BC2001), this analysis aimed to show that the results in the 55Gy group were no more than 50%, corresponding to a non-inferiority margin of 10% absolute difference. With 791 patients, one-sided alpha 0.025 and 1:1 ratio between fractionation groups, the study would have 83% power to conclude non-inferiority. However, compliance with LENT/SOM questionnaires was low, so 600 patients with data available would give 71% power to exclude such an absolute difference.

All patients in the BCON and BC2001 trials who received at least one fraction of radiotherapy and for whom data on the fractionation schedule was available were included in the meta-analysis. Summaries of baseline characteristics were tabulated by fractionation schedule. Since patients were not randomised to a fractionation schedule, baseline imbalance was expected and investigated using standardised differences. Any variables with a standardised difference greater than 10% were considered potential confounders and accounted for in the meta-analysis.

Median follow-up and number of events for the time-to-event endpoints ILRC and OS were summarised. For each endpoint, a crude analysis to estimate the relative difference (hazard ratio, HR) between fractionation schedules was first performed in the combined dataset by fitting a stratified Cox proportional hazards model with fractionation schedule as the predictor, a frailty term to account for site clustering and stratifying by trial. The latter incorporated the variability between trials as a fixed factor in the model, specifying trial-specific baseline hazard functions, and assuming proportional hazards within each trial. The frailty term for site was added because fractionation schedules were chosen due to local preferences, therefore it was possible that participants treated at the same hospital were more similar in respect to other factors, including unmeasured ones. An adjusted HR for fractionation effect was fitted using a similar model, but incorporating the trial(s) intervention (whether patients received a concurrent radiosensitiser or not), pre-specified prognostic factors and any variable identified as potential confounder due to baseline imbalance, or showing association (p-value<0.05) with the time-to-event endpoint in a univariate analysis. Pre-specified prognostic factors for ILRC were age, sex, tumour stage, use of neoadjuvant chemotherapy and extent of resection; for OS, age and sex were considered.[5] Assumptions of the model were assessed by graphical assessment of residuals. A likelihood ratio test for heterogeneity of fractionation effect across trials was performed by considering an extended model which included the interaction of

fractionation schedule and trial. Under the null hypothesis of no heterogeneity between trials, the likelihood ratio statistic followed approximately a chi-square distribution with number of trials-1 degrees of freedom.[6]

The number of patients experiencing grade three or greater GI/GU toxicity within five years was summarised by fractionation schedule overall, and at each time point. Toxicities reported at or after three months prior to first recurrence or bladder cancer death were treated as missing to avoid interpreting recurrence symptoms as toxicities. The absolute risk difference (RD) between fractionation schedules in having grade three or higher GI/GU toxicity over five years was estimated using a generalized linear binomial model and a random intercept for centre, to account for clustering within sites.[7] A crude model was first fitted with fractionation schedule and including trial as a fixed effect. Parameters of the model were estimated under the generalised estimating equations (GEE) framework: sandwich estimators of the standard errors were produced assuming an exchangeable structure for the working correlation structure, as it assumed equal correlation between any two patients within the same site and that patients from different sites are independent. These estimates were corrected by a sampling correction factor of $J/(J-p-1)$ (J is the number of centres and p is the number of variables in the model) to account for the small number of centres in the data.[8] In the adjusted analysis, we also included the trial(s) intervention, age, sex, and any confounders that were identified as imbalanced at baseline, or associated to the toxicity endpoint in univariate analyses. Heterogeneity between trials was explored considering an interaction effect between fractionation schedule and trial.

Pre-planned subgroup analyses included exploring the fractionation effect within trial and within patients who received radiotherapy alone: a 1% significance level was used in these analyses.

The effect of fractionation schedule on HRQoL was explored in the BC2001 trial only, employing similar methods as used for the trial's HRQoL substudy.9 FACT-BL scores were summarised at baseline, end of trial, 1 and 5 years. Mean difference between fractionation schedules in change from baseline at end of treatment and at one year for the Total (Total), bladder cancer specific (BLCS) and Trial Outcome Index (TOI, sum of BLCS plus physical and functional sub-scales) scores were estimated by analysis of covariance (ANCOVA) regression models, adjusting for trial intervention, baseline score, age, sex, stage and grade. A 1% significance level and corresponding 99% confidence intervals was used to account for multiple time points and subscales.

The risk of bias in the two trials included in the meta-analysis was assessed using a tool developed by the Cochrane collaboration.[10]. Because both trials were unblinded, this was thought to have a potential impact on outcome assessment and reporting. However, the intervention under investigation in this analysis is not the same as for either trial and hence unblinding of randomised treatment is unlikely to bias the effect of fractionation schedule. Therefore, the risk would be judged as low-risk in terms of this analysis. Patients were not randomised to fractionation schedule, so it was expected that fractionation groups would be unbalanced with respect to both subject- and centre-level variables within trial and that confounding may be present. This was accounted for in the analysis by adjusting for the relevant covariates.

References

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4 Analysis of Invasive Loco-regional Control

Table S1. Fractionation effect (55Gy/20f vs 64Gy/32f) in Invasive Loco-Regional Control - crude and adjusted Cox proportional hazards models

	Crude				Adjusted			
	64Gy Ev/pts	**55Gy Ev/pts**	**HR (55Gy to 64Gy ref)**	**95% CI**	**64Gy Ev/pts**	**55Gy Ev/pts**	**HR (55Gy to 64Gy ref)**	**95% CI**
BC2001[1]	67/279	34/177	0.82	(0.54, 1.24)	67/275	31/174	0.67	(0.42, 1.06)
BCON[2]	39/97	78/229	0.85	(0.58, 1.24)	38/94	73/217	0.77	(0.50, 1.19)
Combined one-stage IPD meta-analysis[3]	106/376	112/406	0.83	(0.63, 1.10)	105/369	104/391	0.71	(0.52, 0.96)
Subgroups:								
Received Radiotherapy only[4]	68/216	69/219	0.84	(0.59,1.21)	68/213	64/209	0.72	(0.49,1.05)
Received RT + radiosensitiser[4]	38/160	43/187	0.81	(0.51, 1.27)	37/156	40/182	0.68	(0.42, 1.11)

HR – hazard ratio, CI – confidence interval

[1] *Adjusted for age, sex, randomised treatment, extent of resection, tumour stage, residual mass after resection and neoadjuvant chemotherapy*

[2] *Adjusted for age, sex, randomised treatment, extent of resection, tumour stage and haemoglobin*

[3] *Adjusted for age, sex, , randomised treatment, extent of resection, tumour stage, haemoglobin and neoadjuvant chemotherapy; model stratified by trial and random effect for centre*

[4] *Adjusted for age, sex, extent of resection, tumour stage, haemoglobin and neoadjuvant chemotherapy; model stratified by trial and random effect for centre*

Table S2. Combined one-stage IPD meta-analysis model for Invasive Loco-Regional Control – full adjusted Cox model

Variable		**N. events**	**N. patients**	**HR**	**95% CI**
Fractionation	55Gy	104	391	0.71	(0.52, 0.96)
Sex	Female	43	147	0.97	(0.68, 1.37)
Age (years)	Mean (SD)	209	760	1.02	(1.00, 1.04)
Randomised treatment	RT + intervention	77	338	0.65	(0.49, 0.87)
Tumour stage	3	39	125	1.21	(0.84, 1.75)
	4	10	26	1.78	(0.93, 3.42)
Extent of resection	Complete	90	383	0.80	(0.55, 1.18)
	Partial	77	245	1.10	(0.75, 1.61)
Neoadjuvant chemo	Yes	22	132	0.62	(0.37, 1.05)
Haemoglobin (g/dl)	Mean (SD)	209	760	0.86	(0.79, 0.93)

5 Analysis of Overall Survival

Table S3. Fractionation effect (55Gy/20f vs 64Gy/32f) in Overall Survival - crude and adjusted Cox proportional hazards models

	Crude				Adjusted			
	64Gy Ev/pts	**55Gy Ev/pts**	**HR (55Gy to 64Gy ref)**	**95% CI**	**64Gy Ev/pts**	**55Gy Ev/pts**	**HR (55Gy to 64Gy ref)**	**95% CI**
BC2001[1]	200/279	130/177	1.06	(0.85, 1.33)	196/275	127/174	0.93	(0.73, 1.19)
BCON[2]	73/97	168/229	0.87	(0.66, 1.15)	71/94	156/217	0.78	(0.58, 1.05)
Combined one-stage IPD meta-analysis[3]	273/376	298/406	0.99	(0.83, 1.18)	267/369	283/391	0.87	(0.72, 1.06)
Subgroups:								
Received Radiotherapy only[4]	161/216	170/219	1.06	(0.84,1.33)	158/213	160/209	0.92	(0.72, 1.18)
Received RT + radiosensitiser[4]	112/160	128/187	0.91	(0.70, 1.20)	109/156	123/182	0.83	(0.62, 1.11)

HR – hazard ratio, CI – confidence interval

1 Adjusted for age, sex, WHO, randomised treatment, extent of resection, tumour stage, and haemoglobin

2 Adjusted for age, sex, randomised treatment, extent of resection, tumour stage and haemoglobin

3 Adjusted for age, sex, randomised treatment, extent of resection, tumour stage, haemoglobin; model stratified by trial and random effect for centre

4 Adjusted for age, sex, extent of resection, tumour stage, haemoglobin; model stratified by trial and random effect for centre

Table S4. Combined one-stage IPD meta-analysis model for Overall Survival– full adjusted model

Variable		**N. events**	**N. patients**	**HR**	**95% CI**
Fractionation	55Gy	283	391	0.87	0.72, 1.06
Sex	Female	99	147	0.84	0.67, 1.05
Age (years)	Mean (SD)	550	760	1.04	1.03, 1.05
Randomised treatment	RT + intervention	232	338	0.83	0.70, 0.98
Tumour stage	3	97	125	1.13	0.89, 1.43
	4	21	26	1.49	0.95, 2.34
Extent of resection	Complete	277	383	0.89	0.70, 1.14
	Partial	178	245	1.08	0.83, 1.39
Haemoglobin (g/dl)	Mean (SD)	550	760	0.89	0.85, 0.94

6 Analysis of Bladder Specific Survival

Of the 456 BC2001 patients included in the analysis, 230 (50.4%) died due to bladder cancer (49.8% 64Gy, 51.4% 55Gy), 100 (21.9%) died due to other causes (21.9% 64Gy, 22.0% 55Gy). Median follow-up for bladder cancer deaths was 104 months (IQR 71-121), and median follow-up for deaths due to other causes was 135 (IQR 87-NE).

Of the 326 patients in the BCON trial, 144 (44.2%) died due to bladder cancer (51.6% 64Gy, 41.1% 55Gy), 97 (29.8%) died due to other causes (23.7% 64Gy, 32.3% 55Gy). Median follow-up for bladder cancer deaths was 95 months (IQR 60-142), and median follow-up for deaths due to other causes was 131 (73-NE).

In BCON, cause of death was collected while on active follow-up for the study, but not consistently during retrospective data collection of long-term follow-up. For this reason, we have estimated the fractionation effect for bladder cancer specific survival (BCSS) within 10 years (patients alive by 10 years are censored at t=10). A competing risks analysis was performed to analyse (BCSS).

Figure S1 - Cumulative Incidence of bladder-cancer specific mortality (left) and due to other causes (right) by fractionation schedules in BC2001 and BCON trials

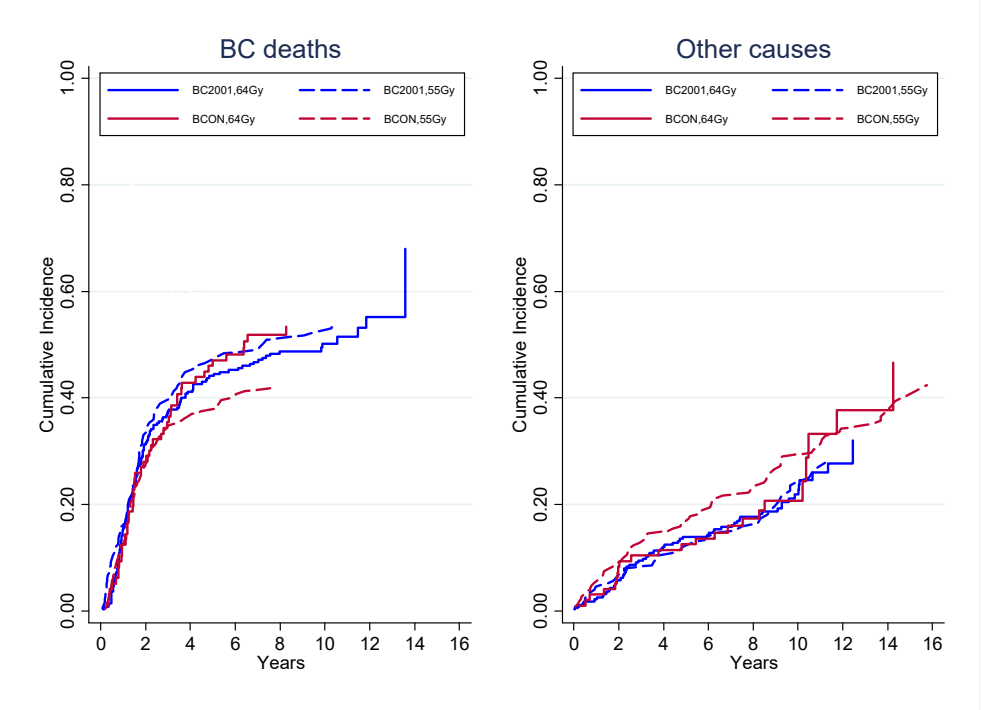


Table S5. Fractionation effect (55Gy/20f vs 64Gy/32f) in Bladder Cancer Specific Survival - crude and adjusted Fine&Gray model

	Crude				Adjusted			
	64Gy Ev/pts	**55Gy Ev/pts**	**HR (55Gy to 64Gy ref)**	**95% CI**	**64Gy Ev/pts**	**55Gy Ev/pts**	**HR (55Gy to 64Gy ref)**	**95% CI**
BC2001[1]	139/279	91/177	1.04	(0.76, 1.41)	133/267	82/162	0.93	(0.72, 1.20)
BCON[2]	50/97	94/229	0.87	(0.58, 1.30)	50/97	93/228	0.80	(0.52, 1.21)
Combined one-stage IPD meta-analysis[3]	189/376	185/406	0.97	(0.76, 1.25)	185/369	174/391	0.83	(0.66, 1.05)

HR – sub-distribution hazard ratio (Fine&Gray model, CI – confidence interval
1 Adjusted for age, sex, WHO, trial intervention, extent of resection, residual mass post-resection, tumour stage, haemoglobin
2 Adjusted for age, sex, trial intervention, extent of resection, tumour stage and haemoglobin
3 Adjusted for trial, age, sex, trial intervention, extent of resection, tumour stage, haemoglobin

7 Analysis of Toxicity

In the BC2001 trial, the proportion of patients with no toxicity data for analysis (either not collected or with any data collected after 3-months prior of a recurrence and thus censored) was greater in the 55Gy group (32.2%) than in the 64Gy group (27.3%). Amongst those with available toxicity data for analysis, 14% recurred (median 60 months), and 65% died (median 79 months); while for patients with no toxicity data available for analysis, 42% recurred (median 5.8 months), and 90% died (median 10.2 months). In the BCON trial, the proportion of patients with all missing or censored toxicity data was similar in the two groups (55Gy 23.6% vs 64Gy 22.7%). Amongst those with available toxicity data for analysis, 27% recurred (median 48 months), and 67% died (median 73 months); while for patients with no available toxicity data for analysis, 64% recurred (median time to recurrence 5.9), and 94% died (median survival time 8.5).

Table S6. Summary of baseline characteristics in BC2001, BCON and the combined dataset in the toxicity analysis population

		BC2001				BCON				COMBINED BC2001&BCON			
Variable		**N**	**64Gy (n=203)**	**55Gy (n=120)**	**Std diff (%)**	**N**	**64Gy (n=75)**	**55Gy (n=175)**	**Std diff (%)**	**N**	**64Gy (n=278)**	**55Gy (n=295)**	**Std diff (%)**
Sex	Male	323	171 (84.2)	94 (78.3)	15.2	250	63 (84.0)	141 (80.6)	9	573	234 (84.2)	235 (79.7)	11.7
Age (years)	Mean (SD)	323	71.3 (8.7)	71.2 (7.7)	2.3	250	72.1 (8.2)	72.9 (7.9)	9.7	573	71.5 (8.6)	72.2 (7.9)	7.7
Randomised treatment	RT + intervention	323	82 (40.4)	49 (40.8)	1.0	250	42 (56.0)	87 (49.7)	12.6	573	124 (44.6)	136 (46.1)	3.0
Tumour stage	1	323	1 (0.5)	0 (0.0)	47.5	250	11 (14.7)	11 (6.3)	36	573	12 (4.3)	11 (3.7)	40.2
	2		184 (90.6)	89 (74.2)			53 (70.7)	120 (68.6)			237 (85.3)	209 (70.9)	
	3		15 (7.4)	28 (23.3)			9 (12.0)	38 (21.7)			24 (8.6)	66 (22.4)	
	4		3 (1.5)	3 (2.5)			2 (2.7)	6 (3.4)			5 (1.8)	9 (3.1)	
Grade	1	322	1 (0.5)	0 (0.0)	10.6	250	0 (0)	0 (0)	6.4	572	1 (0.4)	0 (0.0)	9.2
	2		28 (13.8)	15 (12.6)			12 (16.0)	24 (13.7)			40 (14.4)	39 (13.3)	
	3		174 (85.7)	104 (87.4)			63 (84.0)	151 (86.3)			237 (85.3)	255 (86.7)	
Extent of resection	Biopsy/ Not resected	320	15 (7.5)	14 (11.8)	36.1	243	18 (24.0)	49 (29.2)	15.4	563	33 (12.0)	63 (22.0)	40.2
	Complete		138 (68.7)	61 (51.3)			33 (44.0)	62 (36.9)			171 (62.0)	123 (42.8)	
	Partial		48 (23.9)	44 (36.9)			24 (32.0)	57 (33.9)			72 (26.0)	116 (35.2)	
Neoadjuvant chemo	Yes	323	51 (25.1)	43 (35.8)	23.4	250	0 (0.0)	0 (0.0)	0	573	51 (18.4)	43 (14.6)	10.2
Haemoglobin (g/dl)	Mean (SD)	323	13.2 (1.8)	12.7 (1.8)	28.5	247	14.0 (1.5)	13.7 (1.5)	17.7	570	13.4 (1.8)	13.3 (1.7)	6.6

Table S7. Fractionation effect (55Gy/20f vs 64Gy/32f) in toxicity - crude and adjusted crude and adjusted binary models estimating the average difference between fractionation groups in absolute risk of experiencing a grade 3/4 bladder or rectum toxicity within 5 years after treatment

	Crude				Adjusted			
	64Gy Ev/pts	**55Gy Ev/pts**	**Risk difference (55Gy – 64Gy)**	**95% CI**	**64Gy Ev/pts**	**55Gy Ev/pts**	**Risk difference (55Gy – 64Gy)**	**95% CI**
BC2001[1]	62/203	31/120	-4.79	(-15.06, +5.47)	62/203	31/120	-5.24	(-15.78, +5.30)
BCON[1]	27/75	66/175	-0.84	(-15.39, +13.71)	27/75	66/175	-0.79	(-17.84, +16.27)
Combined one-stage IPD meta-analysis[2]	89/278	97/295	-2.88	(-11.15, +5.39)	89/278	97/295	-3.37	(-11.85, +5.10)
Subgroups:								
Received Radiotherapy only[3]	57/154	46/159	-10.81	(-22.16, +0. 55)	57/154	46/159	-12.51	(-23.84, -1.19)
Received RT + radiosensitiser[3]	32/124	51/136	+6.67	(-5.42, +18.76)	32/124	51/136	+7.32	(-5.03, +19.67)

CI – confidence interval

1 Adjusted for age, sex, and randomised treatment

2 Adjusted for age, sex, randomised treatment and trial; randomised intercept for centre

3 Adjusted for age, sex and trial

Table S8. Combined one-stage IPD meta-analysis model for late toxicity – full adjusted model

Variable		**% Risk Difference**	**95% CI**
Fractionation	55Gy – 64Gy	-3.37	-11.85, +5.10
Sex	Female – Male	+13.90	+2.52, +25.27
Age (years)	1 g/dL	-0.09	-0.59, +0.41
Trial intervention	RT + radiosensitiser – RT alone	-1.40	-9.43, +6.63,
Trial	BCON-BC2001	+9.05	+0.30, +17.81

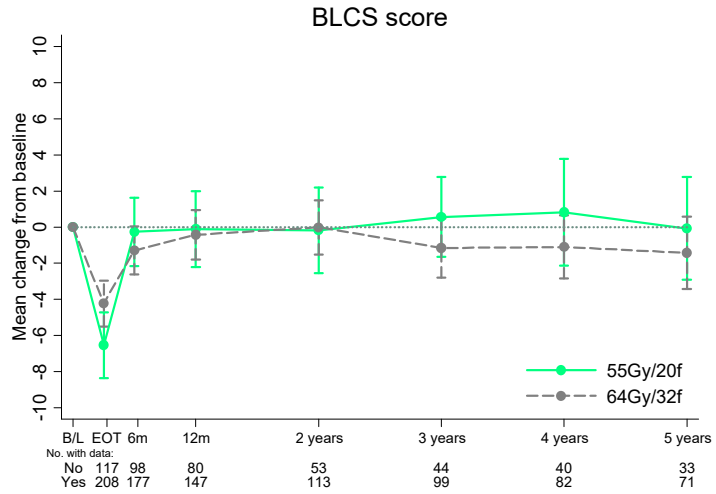
8 Analysis of Health-Related Quality of life

Table S9. BC2001: General FACT-BL scores per subscale and timepoint, by fractionation schedule

	Baseline			EOT			1 year			5 years		
	N	**Median**	**Q1-Q3**	**N**	**Median**	**Q1-Q3**	**N**	**Median**	**Q1-Q3**	**N**	**Median**	**Q1-Q3**
55Gy/20f												
BLCS	165	34	29-38	126	26	22-33	87	34	30-38	33	34	30-38
TOTAL	167	123	106-134	126	114	90-125	86	125	111-135	33	129	115-137
TOI	165	79	70-87	124	69	52-79	86	80	70-87	33	83	71-87
EWB	166	20	17-22	127	21	18-24	86	22	19-23	33	23	19-24
FWB	167	21	17-26	125	18	13-23	86	21	17-25	34	24	14-27
SWB	165	25	22-27	125	25	21-28	87	25	22-28	32	24	20-28
PWB	168	25	21-27	127	23	17-26	87	26	22-27	34	26	21-27
64Gy/32f												
BLCS	256	35	29-39	223	31	25-35	155	35	31-39	76	35	31-37
TOTAL	254	125	109-133	223	116	99-131	154	127	116-138	74	127	116-135
TOI	253	81	69-88	222	73	60-84	154	83	72-92	74	82	76-89
EWB	254	20	17-22	225	21	19-23	156	21	19-23	75	22	20-24
FWB	254	21	17-25	225	20	14-24	156	23	17-26	75	22	18-26
SWB	250	25	22-28	220	24	22-27	155	25	21-27	73	24	21-27
PWB	255	25	22-27	224	24	20-26	155	26	24-27	74	26	24-28

BLCS= Bladder cancer subscale; EWB=Emotional well-being; FWB= Functional well-being; SWB=Social well-being; PWB= Physical well-being; TOI=Trial Outcome Index (PWB+FWB+BLCS)

Figure S2. Health-Related Quality of Life in BC2001: mean change from baseline (with 99% confidence intervals) in Bladder Cancer Specific Scale (BLCS), Trial Outcome Index (TOI) and TOTAL scores (TOI=BLCS+PWB+FWB)



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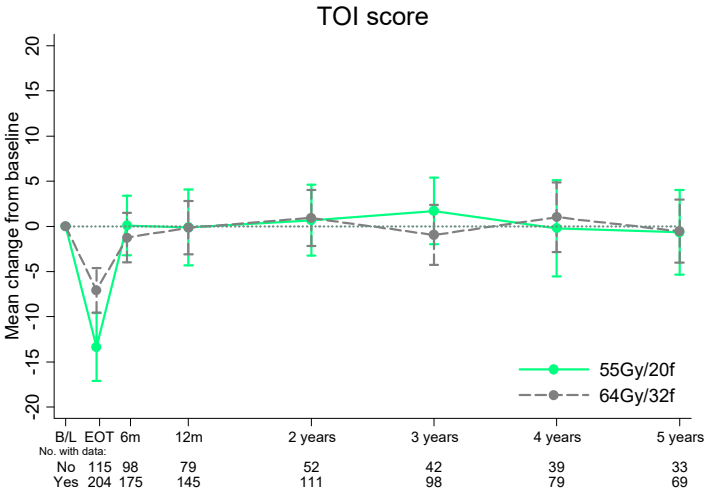

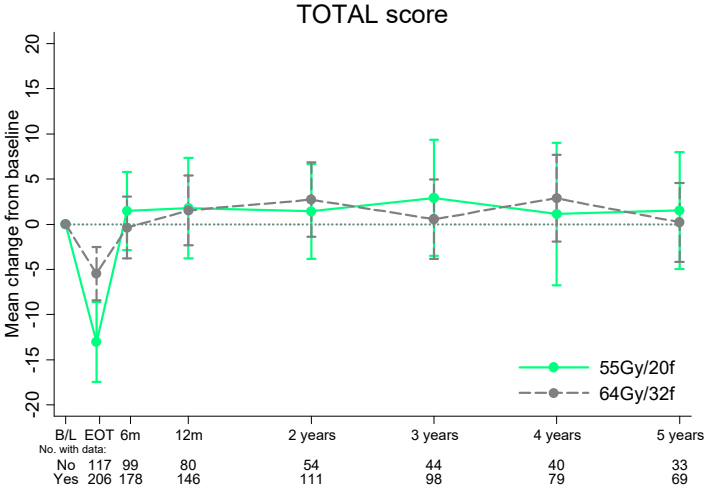

9 Radiobiology of hypofractionation - methods

Conventional and hypo-fractionated treatment regimens can be compared using Biologically Effective Dose (BED) and an equation that includes the effect of treatment time:

$$BED = D * \left[1 + d * {}^{\alpha}/_{\beta}\right] - \gamma(T - Tk)$$

Where D is the total dose, d is the dose per fraction, α/β is a biological parameter that describes the sensitivity to fraction size, γ is a time factor representing the loss of dose per day due to repopulation, T is the overall treatment time and Tk is the kick off time for repopulation.

For normal tissues it is usual to apply the BED formula without the term for repopulation:

$$BED = D * \left[1 + d * {}^{\alpha}/_{\beta}\right]$$

Bladder cancer is considered a rapidly proliferating cancer with an α/β of 10Gy[1] and there is evidence to suggest a loss (γ) of 0.2-0.36 Gy per day after approximately 5 weeks of treatment due to repopulation.[2] Using α/β of 10 Gy without accounting for overall time suggests that 66Gy/32f and 55Gy/20f have Biologically Effective Dose (BED) of 76.8Gy and 70.1Gy respectively. This difference was reduced when a time factor was included, with the maximum reduction for kick-off time (Tk) of 28 days or less. If BED was calculated with γ=0.36 and Tk=28 days, the 64Gy/32f and 55f/20f have BED of 71Gy and 70.1Gy respectively.

Estimates for α/β ratios for late reactions in human bladder range from 3-7.[3] Using a commonly accepted value of 5, the BED for late reactions for 64Gy/32f and 55Gy/20f was 89.6Gy and 85.3Gy respectively, indicating that the longer 2Gy fractionation schedule is marginally 'hotter' than the shorter 20 fraction schedule. It should be noted that using an α/β of 3Gy makes the fractionation schedules equivalent. Also, there was evidence for a time-dependence due to consequential injury from early reactions which reduces the BED for the 64Gy/32f and consequently produces equivalent BED values for late reactions from both fractionation schemes.

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