Clinical Trial of Oral Nelfinavir Before and During Radiation Therapy for Advanced Rectal Cancer

Esme J. Hill[1], Corran Roberts[2], Jamie M. Franklin[1], Monica Enescu[3], Nicholas West[4], Thomas P. MacGregor[1], Kwun-Ye Chu[1], Lucy Boyle[5], Claire Blesing[6], Lai-Mun Wang[6], Somnath Mukherjee[1], Ewan M. Anderson[6], Gina Brown[7], Susan Dutton[2], Sharon B. Love[2], Julia A. Schnabel[3], Phil Quirke[4], Ruth Muschel[1], William G. McKenna[1], Michael Partridge[1], Ricky A. Sharma[1]

1. Oxford Cancer Imaging Centre and NIHR Oxford Biomedical Research Centre, Department of Oncology, University of Oxford, Old Road Campus Research Building, Oxford OX3 7DQ, UK
2. Centre for Statistics in Medicine, Nuffield Department of Orthopaedics Rheumatology and Musculoskeletal Sciences, University of Oxford, Windmill Road, Oxford OX3 7LD, UK
3. Institute of Biomedical Engineering, Department of Engineering Science, University of Oxford, Old Road Campus Research Building, Oxford OX3 7DQ, UK
4. Section of Pathology and Tumour Biology, Leeds Institute of Cancer & Pathology, University of Leeds, Level 4, Wellcome Trust Brenner Building, St James's University Hospital, Leeds LS9 7TF, UK
5. Oncology Clinical Trials Office (OCTO), Department of Oncology, University of Oxford, Old Road Campus Research Building, Oxford OX3 7DQ, UK
6. Oxford University Hospitals NHS Trust, Churchill Hospital, Old Road, Oxford OX3 7LE, UK
7. Radiology Department, Royal Marsden Hospital, Downs Road, Sutton, Surrey SM2 5PT, UK

Corresponding author: R A Sharma, CRUK-MRC Oxford Institute for Radiation Oncology, Department of Oncology, Old Road Campus Research Building, Oxford, OX3 7DQ, UK; E-mail: ricky.sharma@oncology.ox.ac.uk; Tel : +44 1865 617322

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Statement of translational relevance

Nelfinavir, a PI3-kinase pathway inhibitor, is a radiosensitizer which increases tumor blood flow in preclinical models. This early-phase study demonstrates the safety of nelfinavir combined with radiation therapy (RT) for rectal cancer. It includes the development of imaging biomarkers of tumor perfusion and a tissue biomarker of radiosensitization which can be measured in biopsy tissue taken before and after treatment. Based on the results of this study, the efficacy of nelfinavir-RT versus RT alone merits phase II evaluation in the treatment of rectal cancer, including measurement of tumor blood flow.

Abstract

Purpose

Nelfinavir, a PI3-kinase pathway inhibitor, is a radiosensitizer which increases tumor blood flow in preclinical models. We conducted an early-phase study to demonstrate the safety of nelfinavir combined with hypofractionated radiotherapy (RT) and to develop biomarkers of tumor perfusion and radiosensitization for this combinatorial approach.

Patients and Methods

Ten patients with T3-4 N0-2 M1 rectal cancer received 7 days of oral nelfinavir (1250 mg bd) and a further 7 days of nelfinavir during pelvic RT (25 Gy/5 fractions/7 days). Perfusion CT (p-CT) and DCE-MRI scans were performed pre-treatment, after 7 days of nelfinavir and prior to last fraction of RT. Biopsies taken pre-treatment and 7 days after the last fraction of RT were analysed for tumor cell density (TCD).

Results

There were 3 drug-related grade 3 adverse events: diarrhea, rash, lymphopenia. On DCE-MRI, there was a mean 42% increase in median K_{trans}, and a corresponding median 30% increase in mean blood flow on p-CT during RT in combination with nelfinavir. Median TCD decreased from 24.3% at baseline to 9.2% in biopsies taken 7 days after RT (P=0.01). Overall, 5/9 evaluable patients exhibited good tumor regression on MRI assessed by Tumor Regression Grade (mrTRG).

Conclusions

This is the first study to evaluate nelfinavir in combination with RT without concurrent chemotherapy. It has shown that nelfinavir-RT is well tolerated and is associated with increased blood flow to rectal tumors. The efficacy of nelfinavir-RT versus RT alone merits clinical evaluation, including measurement of tumor blood flow.

Introduction

Pelvic radiotherapy (RT) has an important role in the treatment of patients with rectal adenocarcinoma. Short course RT, 25 Gy delivered in 5 daily fractions in one week followed by surgery within 5-7 days, can halve the risk of local recurrence in patients with operable rectal cancer(1, 2). Long-course pre-operative chemo-radiotherapy (LCCRT), typically 45-50.4 Gy in 25-28 daily fractions over 5-6 weeks in combination with 5-fluorouracil or capecitabine as a radiosensitizer, is generally offered to patients with locally advanced tumors. Tumor regression has been shown to correlate with improved outcomes for patients(3-5).

The optimal first treatment for patients with a symptomatic primary rectal cancer and distant metastases at presentation is a matter of debate. Systemic therapy is not effective in all patients; although it may achieve response after 6-8 weeks of therapy, it does not provide rapid symptom relief for all patients(6). Planning and delivery of LCCRT may delay delivery of full-dose systemic therapy and may therefore compromise surgical treatment of metastatic disease (e.g. liver surgery for operable metastases). A strategy of short-course RT followed 2 weeks later by full-dose systemic combination chemotherapy can be used to prevent this delay. Short-course RT can safely precede full-dose systemic therapy (e.g. capecitabine and oxaliplatin and bevacizumab), resulting in pathological complete response (pCR) rates above 25% and radical resection and/or radiofrequency ablation of all metastatic disease in the majority of patients(7).

One factor increasing cellular resistance to RT is over-expression of activated oncogenes, such as the epidermal growth factor receptor (EGFR)(8), RAS(9) or loss of the tumor suppressor gene PTEN(10). These mutations share molecular signaling via the phosphatidylinositol 3-kinase (PI3K)-Akt-pathway. We have previously shown that inhibition of this pathway augments response to RT *in vitro* and *in vivo* in cells with constitutive activation of this pathway, an effect not seen in cells with a non-activated pathway(11-14). This pathway is frequently altered in humans with colorectal cancer (CRC)(15). Since the PI3K signaling pathway can be constitutively activated in tumor cells, yet not in host cells, an inhibitor of this pathway might be expected to improve the therapeutic index through selective tumor radiosensitization(16).

Nelfinavir is an HIV-protease inhibitor (HPI) which has been shown to inhibit Akt at standard clinical doses and to cause radiosensitization *in vivo(17)*. In addition to intrinsic radiosensitization, we have shown previously that nelfinavir caused sustained improvements in tumor perfusion and reduction in hypoxia in a mouse xenograft model(18). Although some clinical studies have investigated nelfinavir in combination with chemoradiotherapy (see Table 1), there are no published data on the addition of nelfinavir to RT without concomitant chemotherapy. Nor are there data on whether the changes in perfusion observed in pre-clinical studies with nelfinavir are replicated in human subjects with cancer. Dynamic contrast-enhanced MRI (DCE-MRI) and perfusion CT (p-CT) have previously been used to detect changes in tumor perfusion induced by anti-angiogenic drugs(19, 20) and chemoradiotherapy for rectal cancer(21-25).

A barrier to the advancement of radiosensitizers is uncertainty regarding the optimal primary endpoint for clinical trials. Endpoints traditionally used, such as pCR rate, radiological response or disease free survival, have a number of limitations, including variability of definitions(26). The development of new tissue biomarkers of response is highly desirable for the evaluation of novel radiosensitizers. We have developed a quantitative assessment of tumor cell density (TCD) which is a predictor of survival in patients with CRC(27). We are currently exploring this technique to compare different pre-operative RT schedules(28).

The objective of the SONATINA (Study Of Nelfinavir Addition to Radiotherapy Treatment In Neo-Adjuvant Rectal Cancer) clinical trial was to investigate the safety of Nelfinavir administered before and during RT in patients with rectal adenocarcinoma. We also explored the feasibility of incorporating biomarkers of RT that could be used in efficacy studies and the ability of p-CT and DCE-MRI to detect changes in tumor perfusion during therapy.

Patients and Methods

Study design

SONATINA was a non-randomized, open-label clinical trial (EudraCT number: 2010-020621-40) to establish the safety of nelfinavir with hypofractionated pelvic RT. The primary outcome was measured by the occurrence of any grade 3 or higher toxicities (Common Terminology Criteria for Adverse Events (CTCAE), version 4.0) within 28 days of the last fraction of RT. Since the primary outcome was the safety of this novel combinatorial therapy, there was no control group. Secondary outcomes included radiological response of primary tumor at 8 weeks post RT, feasibility of measuring a tissue biomarker (TCD) in pre-treatment biopsies and biopsies taken 7 days after RT, and feasibility of using dynamic imaging to evaluate tumor perfusion.

Ethical approval was obtained from National Research Ethics Service Committee South Central (reference 10/H0604/61). Key inclusion criteria were patients with histologically-proven adenocarcinoma of the rectum, radiological evidence of M1 disease, suitability for short-course RT as primary treatment (determined by Colorectal Tumor Board), ECOG performance status 0-2, and age ≥ 18 years. Exclusion criteria included previous pelvic RT, recent severe cardiac disease or operable primary tumor (in opinion of Tumor Board).

Treatment

Patients received 7 days of oral Nelfinavir (1250 mg bd) before RT and a further 7 days of nelfinavir during RT. This dose of nelfinavir has been shown to consistently reduce levels of Akt phosphorylation in peripheral-blood mononuclear cells in patients with cancer (29). Compliance logs were used to check that all doses were taken as prescribed. The total dose of RT was 25 Gy, delivered in 5 Gy fractions on 5 days during a 7-day period as a single-phase treatment prescribed to the International Commission on Radiation Units (ICRU) Reference Point. The dose constraints were set such that at least 99% of the planning target volume (PTV) should receive 95% of the prescription dose. The PTV maximum was no more than 107% of the prescribed dose to the ICRU reference point. For all patients, 3-7 photon beams (6 or 15 MV) were used, with the entire plan displayed in physical dose. Conformal RT plans were reviewed by a RT quality assurance panel

(independent clinician, radiographer, physicist) prior to delivery of the first fraction. Verification imaging by cone beam CT to localize the treatment volume was required prior to every fraction for the first 3 fractions. In order to treat metastases, patients were permitted to commence systemic chemotherapy 14 days after completion of RT.

Details of procedures

Patients underwent MRI of the pelvis at baseline and 8 weeks after completion of RT for assessment of Tumor Regression Grade (mrTRG) according to a recognized scoring system(30). As previously published(30), patients with mrTRG score of 1-3 on MRI scan were classified as having 'good mrTRG score' and patients with mrTRG score of 4 or 5 were classified as having 'poor mrTRG score'. Anonymised scans were assessed by 2 independent radiologists; agreement was evaluated by weighted Kappa statistic. In cases of discrepancy, scans were assessed by a third independent radiologist and consensus derived.

Dynamic imaging

In order to explore changes in tumor perfusion induced by protocol therapy, DCE-MRI and p-CT scans of the rectum were incorporated at 3 timepoints: before commencement of nelfinavir, the day before commencement of RT (i.e. Day 7 of nelfinavir) and on the last day of treatment (before the RT fraction was delivered). Mean p-CT parameters [Blood Flow (BF), Blood Volume (BV) and Mean Transit Time (MTT)] and median DCE-MRI parameters (K^{trans}, K^{ep} and V^{e}) scans were measured in the tumour volume of interest were and percentage change in these values were presented graphically.

Tissue biomarkers

In diagnostic biopsies and biopsies performed 7 days after completion of RT, TCD was measured in digitally scanned hematoxylin and eosin stained slides using an automated scanning system (Aperio XT, Aperio Technologies, Vista CA) at 200x magnification(27, 28). In cases where there was variation in TCD across the specimen, we used the area of tumor with highest TCD, as we have previously reported and correlated with clinical outcomes(27). Immunohistochemistry was carried out on pre-treatment rectal biopsy specimens using the Leica Bond-Max

automated immunostainer (Leica Microsystems, Wetzlar, Germany) on 5 µM sections cut from formalin-fixed paraffin-embedded tissue. As an indicator of baseline characteristics, pre-treatment biopsy sections were stained for the following biomarkers: CAIX, HIF- 1α, Phospho-PRAS40 (see Supplementary Information).

Statistical analyses

The Wilcoxon Signed Rank test was used to determine pairwise differences for non-parametric data and the paired Student's t-test was used to determine pairwise differences for parametric data.

Results

Recruitment, compliance and toxicities

From April 2011 to August 2013, 19 patients were screened and 10 patients recruited (Figure 1; Table 2). All patients completed RT as per protocol. Compliance logs revealed that one patient missed one dose of nelfinavir and another patient missed two doses of nelfinavir.

There were no Grade 4 toxicities. Two patients stopped taking nelfinavir early because of toxicity: one on day 13 of treatment because of an allergic rash (grade 3, probably related), the other on day 4 due to vomiting (grade 3, possibly related but patient had pre-existing partial gastric outlet obstruction). Additionally, 5 patients had Grade 3 toxicities within 28 days of RT (Table 3). One patient was admitted to hospital with Grade 3 diarrhea 23 days after completion of RT and nelfinavir, which was 7 days after commencement of Oxaliplatin and 5FU chemotherapy. This event was considered to be related to chemotherapy and possibly related to RT, but unrelated to nelfinavir. Another patient developed Grade 3 diarrhea 4 days after completion of nelfinavir and RT; this event was considered to be causally related to protocol therapy. Another patient had Grade 3 perianal pain due to hemorrhoids, probably related to RT.

With regard to laboratory values, one patient developed Grade 3 lymphopenia on the last day of protocol therapy; this persisted on a blood test one month following completion of therapy. The total white cell count was normal and the patient had no evidence of active infection. A number of Grade 1 or 2 abnormalities in liver function tests were observed within 3 months of therapy, likely to be related to liver metastases or chemotherapy (Supplementary Table 1). One patient had hyponatremia (Grade 3) which preceded protocol therapy, and worsened transiently during an episode of diarrhea after RT. Since a known side effect of nelfinavir is diabetes mellitus, fasting glucose was checked during treatment and follow-up. Three patients had Grade 1 or 2 hyperglycemia after 7 days of nelfinavir; blood glucose was normal on subsequent testing 28 days after completion of therapy.

Radiological responses

Using a recognized scoring system(30), inter-observer agreement between two independent radiologists was good, with weighted kappa score of 0.79. Of 9 patients who completed MRI scans of the pelvis 8 weeks after completion of nelfinavir and RT to assess mrTRG response of the primary tumor, 5 patients exhibited "good" tumor regression according the definitions of the scoring system(30) (Table 4 and Supplementary Figure 1). It should be noted that, as discussed in the Introduction, a major benefit of the treatment strategy adopted in this clinical trial was that patients were permitted to commence full-dose systemic chemotherapy to treat metastatic disease as early as 14 days from the last fraction of RT, as documented in Table 4.

Dynamic Imaging

All 10 patients in the study successfully completed p-CT scans at 3 time points (Supplementary Figure 2). The pCT scans for one patient (patient 7) were excluded from analysis for technical reasons. Nine patients underwent DCE-MRI scanning at all 3 timepoints. One patient (patient 1) did not undergo the second DCE-MRI scan because of vertigo. A further 3 scans were excluded from analysis because of inadequate contrast enhancement or contrast extravasation.

Analyzing the percentage change in perfusion parameters between the pre-treatment scans (scan 1) and the scan on the 7^{th} day of nelfinavir (scan 2), the median BF was 37.3 at scan 1, and 43.9 at scan 2 (non-significant by Wilcoxon Signed Rank test). There were also no statistically significant changes in BV or MTT demonstrated between scans 1 and 2 (non-significant by Wilcoxon Signed Rank test).

Between the p-CT on the 7^{th} day of nelfinavir (scan 2) and the scan at the end of RT (scan 3), an increase in BF in association with a decrease in MTT was observed in 8 of 9 evaluable patients (Figure 2A). A significant median 30% increase in BF (p=0.01, Wilcoxon Signed-Rank test) and a 29% median decrease in MTT was observed *(*p=0.01, Wilcoxon Signed-Rank test) on p-CT from scan 2 to scan 3 (Supplementary Table 2).

Between the DCE-MRI on the 7^{th} day of nelfinavir (scan 2) and the scan at the end of RT (scan 3), an increase in median K^{trans} was demonstrated in all 7 evaluable

patients (Figure 2B and Supplementary Table 3). Between scans 2 and 3, there was a 42% (0.08 min^{-1}) mean increase in median K^{trans} and a 13% (0.07) mean increase in median V^{e}. (p=0.03 and p=0.02 respectively, Student's t-test).

Tissue biomarkers

TCD was evaluable in all of the pre-treatment rectal biopsy specimens and in 9 out of 10 post-radiotherapy biopsy specimens (Figure 2C). The median TCD decreased from 24 (interquartile range from 13 to 45) at baseline to 9 (interquartile range 3-16) on post-treatment biopsies. One of the post-treatment biopsies contained adenoma cells but no malignant cells, which was attributed to sampling error; this sample was not included in analyses.

The sample size was not adequate to study potential relationships between somatic or immunohistochemical analyses (Supplementary Figure 3) at baseline and radiological response 8 weeks from the end of RT, but these data are presented in Table 4 and Supplementary Tables 4-6 since they may assist in the design of future studies of this treatment combination. Of note, 7 out of 10 tumours had *KRAS* mutation.

Discussion

Nelfinavir has been shown to inhibit Akt at standard clinical doses and to cause radiosensitization *in vivo(17)*. This early-phase trial was designed to study the safety of nelfinavir with hypofractionated pelvic RT, and to develop both tissue and imaging biomarkers of the potential efficacy of this combinatorial therapy for use in future studies. We have demonstrated that the combination of nelfinavir and hypofractionated pelvic RT is well tolerated in patients with advanced rectal cancer.

Advancement of nelfinavir as a radiosensitizer

Although the sample size in this study was not sufficient to make any definite conclusions about response rate, the proportion of good mrTRG in the study presented here compares favorably to LCCRT for locally advanced rectal cancer. In one large UK study, the rate of good mrTRG for LARC was 50% overall(30) and for ≥T3c tumors only 33%. This compares to 56% in the study presented here, in which 60% patients had T4 tumors and 70% had a *KRAS* mutation. It should be noted that 4 of the patients with good mrTRG score had 3-6 weeks of chemotherapy between the end of RT and MRI assessment. Although systemic therapy may have contributed to the clinical response rates observed, the ability to administer full-dose systemic therapy soon after RT appears to be a promising treatment strategy with regard to clinical response rates. The efficacy of hypofractionated RT followed by systemic chemotherapy in comparison to standard chemo-radiation is currently being tested in the international, multi-centre, randomised trial, RAPIDO (NCT01558921)(31).

Importantly, the SONATINA study is the first clinical trial to assess the safety of nelfinavir and RT without the confounding effect of concurrent chemotherapy (see Table 1). A previous study of nelfinavir and long course chemo-radiotherapy with capecitabine resulted in unacceptable levels of Grade 3 hepatotoxicity(32), which may have been attributable to a drug interaction between chemotherapy and nelfinavir. Similarly, in a study of concurrent nelfinavir, temozolomide and RT for patients with glioma, 3 patients experienced dose-limiting Grade 3 transaminase elevation(33). In our study, we observed 3 Grade 3 toxicities which were considered to be possibly or probably related to nelfinavir: diarrhea, drug rash and lymphopenia. Of these, only the drug rash was a dose-limiting toxicity. Consistent with the

published toxicities of hypofractionated pelvic RT without nelfinavir(34-36), our conclusion is that the addition of nelfinavir to hypofractionated pelvic RT is well tolerated. Importantly, hepatotoxicity was not observed in our study (see Table 1, Supplementary Information). It should be noted that 7 out of 10 patients treated in the clinical trial reported here had low rectal tumors (Table 2); we propose that future studies including patients with mid and high rectal tumors should carefully document toxicities to ensure the safety of treating larger volumes of small intestine with RT.

Dynamic imaging as a biomarker of efficacy

In addition to intrinsic radiosensitization, we have shown previously that nelfinavir caused sustained improvements in tumor perfusion and reduction in hypoxia in a mouse xenograft model after 5-14 days of treatment(18). We therefore evaluated 2 imaging biomarkers to measure potential changes in perfusion during nelfinavir therapy in patients with cancer: p-CT and DCE-MRI. Although no changes were observed from 7 days of the trial drug, our study showed a 30% increase in mean BF using p-CT and a 42% mean increase in median k^{trans} using DCE-MRI scans during RT and nelfinavir. The intra-subject coefficient of variation for BF in colorectal tumors has been reported to be in the range 14-23%(37, 38) and studies suggest that the coefficient of variation for k^{trans} measurements in tumors using DCE-MRI is of the order of 20%(39, 40). In our study, the consistency between the findings of the 2 imaging modalities adds substantial support to the observation of increased tumor perfusion. Although k^{trans} can be affected by permeability, our findings from p-CT as well as DCE-MRI suggest increased blood flow from the combination of nelfinavir plus RT.

Since there was no control group (i.e. no nelfinavir) in this early-phase trial designed to show the safety of protocol therapy, it is not possible to differentiate the effect of RT on blood flow from the effect of nelfinavir plus RT in the data from our imaging biomarkers. Previous studies of LCCRT have demonstrated increases in tumor perfusion parameters during the initial weeks of RT(22, 41) followed by subsequent decreases in tumor perfusion after completion of therapy(21, 24, 42-44). Our findings are consistent with previously reported increases in median k^{trans} between baseline and the fifth fraction of hypofractionated RT for locally advanced rectal cancer(23). In order to ascertain whether the significant changes we have observed are due to

RT or due to the combination of nelfinavir with RT, we propose that phase II studies of the efficacy of nelfinavir-RT versus RT alone should incorporate imaging biomarkers of blood flow.

Tissue biomarkers

At present, tissue biomarkers for the selection of patients for a treatment strategy including a novel radiosensitizing drug do not exist. Visual estimation of the tumor:stroma ratio has been shown to be prognostic for patients with localized colon cancer (45), but this has not been studied in patients with metastatic rectal cancer scheduled to receive RT. We sought to develop a reproducible, quantitative tissue biomarker of potential radiosensitization for use in future clinical trials. We have previously assessed TCD in pre-treatment biopsy specimens and resected tumors(27, 28), and in the study presented here we assessed the feasibility of measuring TCD in both pre-RT and post-RT biopsy samples obtained at endoscopy.

Our results are in favour of the hypothesis that the addition of nelfinavir to hypofractionated RT may result in additional tumor cell kill compared to RT alone. Compared to our previous study of 45 rectal cancer patients who received 25 Gy in 5 fractions of RT to the pelvis followed by surgery 7 days after the end of radiotherapy(28), whose TCD values ranged from 14 to 46, the range of post-treatment TCDs in this study was 1 to 21. Based on these findings, we conclude that TCD can be measured in biopsies taken pre- and post-RT. Although TCD could be developed further as a biomarker of radiosensitizing drugs for use in prospective clinical trials, there are limitations in assessing TCD from biopsies due to differences in sampling techniques. Larger, correlative studies with imaging such as mrTRG are warranted.

Conclusions

This study has shown that the combination of nelfinavir and hypofractionated RT for locally advanced rectal cancer is well tolerated and that this novel treatment strategy can be followed by combination chemotherapy as early as 14 days after RT to treat metastatic disease. Consistent with previous studies of RT, nelfinavir plus hypofractionated RT significantly increased mean blood flow to tumor compared to baseline values. The tissue biomarker TCD can be measured on biopsies taken

before and after RT; it is a candidate biomarker for systematic development for assessing potential radiosensitizing drugs prior to phase II evaluation.

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Author contributions

Concept and design: All authors

Collection and assembly of data: Esme Hill, Lucy Boyle

Data analysis and interpretation: Esme Hill, Corran Roberts, Susan Dutton, Sharon Love, Ricky Sharma

Manuscript writing: All authors

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References

1. Kapiteijn E, Marijnen CA, Nagtegaal ID, Putter H, Steup WH, Wiggers T, et al. Preoperative radiotherapy combined with total mesorectal excision for resectable rectal cancer. N Engl J Med. 2001;345:638-46.
2. Sebag-Montefiore D, Stephens RJ, Steele R, Monson J, Grieve R, Khanna S, et al. Preoperative radiotherapy versus selective postoperative chemoradiotherapy in patients with rectal cancer (MRC CR07 and NCIC-CTG C016): a multicentre, randomised trial. Lancet. 2009;373:811-20.
3. Theodoropoulos G, Wise WE, Padmanabhan A, Kerner BA, Taylor CW, Aguilar PS, et al. T-level downstaging and complete pathologic response after preoperative chemoradiation for advanced rectal cancer result in decreased recurrence and improved disease-free survival. Dis Colon Rectum. 2002;45:895-903.
4. Bouzourene H, Bosman FT, Seelentag W, Matter M, Coucke P. Importance of tumor regression assessment in predicting the outcome in patients with locally advanced rectal carcinoma who are treated with preoperative radiotherapy. Cancer. 2002;94:1121-30.
5. Vironen J, Juhola M, Kairaluoma M, Jantunen I, Kellokumpu I. Tumour regression grading in the evaluation of tumour response after different preoperative radiotherapy treatments for rectal carcinoma. Int J Colorectal Dis. 2005;20:440-5.
6. Schrag D, Weiser MR, Goodman KA, Gonen M, Hollywood E, Cercek A, et al. Neoadjuvant chemotherapy without routine use of radiation therapy for patients with locally advanced rectal cancer: a pilot trial. J Clin Oncol. 2014;32:513-8.
7. van Dijk TH, Tamas K, Beukema JC, Beets GL, Gelderblom AJ, de Jong KP, et al. Evaluation of short-course radiotherapy followed by neoadjuvant bevacizumab, capecitabine, and oxaliplatin and subsequent radical surgical treatment in primary stage IV rectal cancer. Ann Oncol. 2013;24:1762-9.
8. Bonner JA, De Los Santos J, Waksal HW, Needle MN, Trummel HQ, Raisch KP. Epidermal growth factor receptor as a therapeutic target in head and neck cancer. Semin Radiat Oncol. 2002;12:11-20.
9. McKenna WG, Weiss MC, Bakanauskas VJ, Sandler H, Kelsten ML, Biaglow J, et al. The role of the H-ras oncogene in radiation resistance and metastasis. Int J Radiat Oncol Biol Phys. 1990;18:849-59.
10. Rosser CJ, Tanaka M, Pisters LL, Tanaka N, Levy LB, Hoover DC, et al. Adenoviral-mediated PTEN transgene expression sensitizes Bcl-2-expressing prostate cancer cells to radiation. Cancer Gene Ther. 2004;11:273-9.
11. Gupta AK, McKenna WG, Weber CN, Feldman MD, Goldsmith JD, Mick R, et al. Local recurrence in head and neck cancer: relationship to radiation resistance and signal transduction. Clin Cancer Res. 2002;8:885-92.
12. Grana TM, Rusyn EV, Zhou H, Sartor CI, Cox AD. Ras mediates radioresistance through both phosphatidylinositol 3-kinase-dependent and Raf-dependent but mitogen-activated protein kinase/extracellular signal-regulated kinase kinase-independent signaling pathways. Cancer Res. 2002;62:4142-50.
13. Gupta AK, Bakanauskas VJ, McKenna WG, Bernhard EJ, Muschel RJ. Ras regulation of radioresistance in cell culture. Methods Enzymol. 2001;333:284-90.
14. Kim IA, Fernandes AT, Gupta AK, McKenna WG, Bernhard EJ. The influence of Ras pathway signaling on tumor radiosensitivity. Cancer Metastasis Rev. 2004;23:227-36.

15. Samuels Y, Wang Z, Bardelli A, Silliman N, Ptak J, Szabo S, et al. High frequency of mutations of the PIK3CA gene in human cancers. Science. 2004;304:554.
16. Vivanco I, Sawyers CL. The phosphatidylinositol 3-Kinase AKT pathway in human cancer. Nat Rev Cancer. 2002;2:489-501.
17. Gupta AK, Cerniglia GJ, Mick R, McKenna WG, Muschel RJ. HIV protease inhibitors block Akt signaling and radiosensitize tumor cells both in vitro and in vivo. Cancer Res. 2005;65:8256-65.
18. Qayum N, Muschel RJ, Im JH, Balathasan L, Koch CJ, Patel S, et al. Tumor vascular changes mediated by inhibition of oncogenic signaling. Cancer Res. 2009;69:6347-54.
19. Willett CG, Boucher Y, di Tomaso E, Duda DG, Munn LL, Tong RT, et al. Direct evidence that the VEGF-specific antibody bevacizumab has antivascular effects in human rectal cancer. Nat Med. 2004;10:145-7.
20. O'Connor JP, Rose CJ, Jackson A, Watson Y, Cheung S, Maders F, et al. DCE-MRI biomarkers of tumour heterogeneity predict CRC liver metastasis shrinkage following bevacizumab and FOLFOX-6. Br J Cancer. 2011;105:139-45.
21. Bellomi M, Petralia G, Sonzogni A, Zampino MG, Rocca A. CT perfusion for the monitoring of neoadjuvant chemotherapy and radiation therapy in rectal carcinoma: initial experience. Radiology. 2007;244:486-93.
22. de Vries A, Griebel J, Kremser C, Judmaier W, Gneiting T, Debbage P, et al. Monitoring of tumor microcirculation during fractionated radiation therapy in patients with rectal carcinoma: preliminary results and implications for therapy. Radiology. 2000;217:385-91.
23. Janssen MHM, Aerts HJWL, Kierkels RGJ, Backes WH, Ollers MC, Buijsen J, et al. Tumor perfusion increases during hypofractionated short-course radiotherapy in rectal cancer: Sequential perfusion-CT findings. Radiotherapy and Oncology. 2010;94:156-60.
24. Sahani DV, Kalva SP, Hamberg LM, Hahn PF, Willett CG, Saini S, et al. Assessing tumor perfusion and treatment response in rectal cancer with multisection CT: initial observations. Radiology. 2005;234:785-92.
25. Kierkels RGJ, Backes WH, Janssen MHM, Buijsen J, Beets-Tan RGH, Lambin P, et al. Comparison Between Perfusion Computed Tomography and Dynamic Contrast-Enhanced Magnetic Resonance Imaging in Rectal Cancer. International journal of radiation oncology, biology, physics. 2010;77:400-8.
26. Hill EJ, Nicolay NJ, Middleton MR, Sharma RA. Oxaliplatin as a radiosensitiser for upper and lower gastrointestinal tract malignancies: What have we learned from a decade of translational research? Critical Reviews in Oncology/Hematology. 2012;83:353-87.
27. West NP, Dattani M, McShane P, Hutchins G, Grabsch J, Mueller W, et al. The proportion of tumour cells is an independent predictor for survival in colorectal cancer patients. Br J Cancer. 2010;102:1519-23.
28. West N GH, Treanor D, Sebag-Montefiore D, Thorpe H, Jayne D, Rutten H, Swellengrebel H, Nagtegaal I and Quirke P. Quantitative assessment of tumour cell density in rectal cancer following three pre-operative therapies compared to surgery alone. Journal of Clinical Oncology. 2010;28:3651.
29. Brunner T, Geiger M, Grabenbauer GG, Lang-Welzenbach M, Mantoni TS, Cavallaro A, et al. Phase I trial of the human immunodeficiency virus protease inhibitor nelfinavir and chemoradiation for locally advanced pancreatic cancer. Journal of Clinical Oncology. 2008;26:2699-706.

30. Patel UB, Taylor F, Blomqvist L, George C, Evans H, Tekkis P, et al. Magnetic resonance imaging-detected tumor response for locally advanced rectal cancer predicts survival outcomes: MERCURY experience. J Clin Oncol. 2011;29:3753-60.
31. Nilsson PJ, van Etten B, Hospers GA, Pahlman L, van de Velde CJ, Beets-Tan RG, et al. Short-course radiotherapy followed by neo-adjuvant chemotherapy in locally advanced rectal cancer--the RAPIDO trial. BMC Cancer. 2013;13:279.
32. Buijsen J, Lammering G, Jansen RL, Beets GL, Wals J, Sosef M, et al. Phase I trial of the combination of the Akt inhibitor nelfinavir and chemoradiation for locally advanced rectal cancer. Radiother Oncol. 2013;107:184-8.
33. Alonso-Basanta M, Fang P, Maity A, Hahn SM, Lustig RA, Dorsey JF. A phase I study of nelfinavir concurrent with temozolomide and radiotherapy in patients with glioblastoma multiforme. J Neurooncol. 2014;116:365-72.
34. Hatfield P, Hingorani M, Radhakrishna G, Cooper R, Melcher A, Crellin A, et al. Short-course radiotherapy, with elective delay prior to surgery, in patients with unresectable rectal cancer who have poor performance status or significant co-morbidity. Radiother Oncol. 2009;92:210-4.
35. Pettersson D, Cedermark B, Holm T, Radu C, Pahlman L, Glimelius B, et al. Interim analysis of the Stockholm III trial of preoperative radiotherapy regimens for rectal cancer. Br J Surg. 2010;97:580-7.
36. Radu C, Berglund A, Pahlman L, Glimelius B. Short-course preoperative radiotherapy with delayed surgery in rectal cancer - a retrospective study. Radiother Oncol. 2008;87:343-9.
37. Goh V, Halligan S, Gartner L, Bassett P, Bartram CI. Quantitative colorectal cancer perfusion measurement by multidetector-row CT: does greater tumour coverage improve measurement reproducibility? Br J Radiol. 2006;79:578-83.
38. Goh V, Halligan S, Hugill JA, Bartram CI. Quantitative assessment of tissue perfusion using MDCT: comparison of colorectal cancer and skeletal muscle measurement reproducibility. AJR Am J Roentgenol. 2006;187:164-9.
39. Morgan B, Utting JF, Higginson A, Thomas AL, Steward WP, Horsfield MA. A simple, reproducible method for monitoring the treatment of tumours using dynamic contrast-enhanced MR imaging. Br J Cancer. 2006;94:1420-7.
40. Lankester KJ, Taylor JN, Stirling JJ, Boxall J, d'Arcy JA, Collins DJ, et al. Dynamic MRI for imaging tumor microvasculature: comparison of susceptibility and relaxivity techniques in pelvic tumors. J Magn Reson Imaging. 2007;25:796-805.
41. Kim JH, Kim CK, Park BK, Park SY, Huh SJ, Kim B. Dynamic contrast-enhanced 3-T MR imaging in cervical cancer before and after concurrent chemoradiotherapy. Eur Radiol. 2012;22:2533-9.
42. Lim JS, Kim D, Baek SE, Myoung S, Choi J, Shin SJ, et al. Perfusion MRI for the prediction of treatment response after preoperative chemoradiotherapy in locally advanced rectal cancer. Eur Radiol. 2012;22:1693-700.
43. de Lussanet QG, Backes WH, Griffioen AW, Padhani AR, Baeten CI, van Baardwijk A, et al. Dynamic contrast-enhanced magnetic resonance imaging of radiation therapy-induced microcirculation changes in rectal cancer. Int J Radiat Oncol Biol Phys. 2005;63:1309-15.
44. Curvo-Semedo L, Portilha MA, Ruivo C, Borrego M, Leite JS, Caseiro-Alves F. Usefulness of Perfusion CT to Assess Response to Neoadjuvant Combined Chemoradiotherapy in Patients with Locally Advanced Rectal Cancer. Academic Radiology. 2012;19:203-13.
45. Huijbers A, Tollenaar RA, van Pelt GW, Zeestraten EC, Dutton S, McConkey CC, Domingo E, Smit VT, Midgley R, Warren BF, Johnstone EC, Kerr DJ, Mesker

WE. The proportion of tumor-stroma as a strong prognosticator for stage II and III colon cancer patients: validation in the VICTOR trial. *Ann Oncol*. 2013;24:179-85.
46. Rengan R, Mick R, Pryma D, Rosen MA, Lin L, Maity A et al. A phase I trial of the HIV protease inhibitor nelfinavir with concurrent chemoradiotherapy for unresectable stage IIIA/IIIB non-small cell lung cancer: a report of toxicities and clinical response. J Thorac Oncol. 2012;7:709-15.
47. Bhushan M, Schnabel JA, Risser L, Heinrich MP, Brady JM, Jenkinson M. Motion correction and parameter estimation in dceMRI sequences: application to colorectal cancer. Med Image Comput Comput Assist Interv. 2011;6891:476-83.
48. Rueckert D, Sonoda LI, Hayes C, Hill DL, Leach MO, Hawkes DJ. Nonrigid registration using free-form deformations: application to breast MR images. IEEE Trans Med Imaging. 1999;18:712-21.
49. Rasheed S, Harris AL, Tekkis PP, Turley H, Silver A, McDonald PJ, et al. Assessment of microvessel density and carbonic anhydrase-9 (CA-9) expression in rectal cancer. Pathol Res Pract. 2009;205:1-9.

50. Andersen, J.N., Sathyanarayanan S, Di Bacco A, Chi A, Zhang T, Chen AH et al., Pathway-based identification of biomarkers for targeted therapeutics: personalized oncology with PI3K pathway inhibitors. Sci Transl Med. 2010; 2: p. 43-55.
51. Yan, Y., Serra V, Prudkin L, Scaltriti M, Murli S, Rodríguez O et al., Evaluation and Clinical Analyses of Downstream Targets of the Akt Inhibitor GDC-0068. Clinical Cancer Research 2013; 19: 6976-6986.

Table 1: Summary of clinical studies investigating nelfinavir in combination with chemo-radiation therapy

Study [reference number]	Tumour type	No. of patients	Treatment Regime	Endpoints	G3/4 toxicities observed	Dose Limiting Toxicities	Response rates on CT scans
Brunner et al. (29)	Pancreatic adenocarcinoma (Unresectable or borderline resectable)	12	NFV 1250 mg bd 3 d before and concurrent with : 59.4 Gy pancreas DL1 Cisplatin 30 mg/m² Gemcitabine 200 mg/m² D1,8,22, 29 (n=5) DL2 Cisplatin 30 mg/m² Gemcitabine 300 mg/m² D1,8,22,29 (n=5)	DLT RECIST (CT) response PET response Resection rate	G3 leukopenia (4) G3 neutropenia (3) G3 thrombocytopenia (2) G3 Nausea/vomiting (2) G3(1) G4 (1) Transaminase G3 Bilirubin (2) G3 Alkaline phosphatase (1) G3(2) G4 (1) Infection	G3 upper GI (1) at DL1 G3 nausea and vomiting (1) at DL2	5/10 PR, 6/10 resection, 5/9 CR
Rengan et al. (46)	Non Small Cell Lung Cancer (Unresectable Stage IIIA/IIIB)	16	NFV 7-14 d before and concurrent with: 66.6 Gy in 38# involved field + Cisplatin 50 mg/m² D1, 8, 29, 36 Etoposide 50 mg/m² D1-5 , 29-36 DL1: NFV 625 mg bd (n=5) DL2 : NFV 1250 mg bd (n=8)	DLT CT response PET response	G3 esophagitis (4) G3 pulmonary toxicity (1) G3 leukopenia (3) G3 anemia (2) G3 thrombocytopenia (2) G3 upper GI (3) G3 hypotension (3) G3 fatigue (2) G4 leukopenia (6) G4 thrombocytopenia (1)	None	4/12 CR, 7/12 PR, 1/12 SD
Buijsen et al. (32)	Locally advanced rectal adenocarcinoma	12	50.4 Gy in 28 # pelvis and Capecitabine 825 mg/m² concurrent with NFV: DL1 NFV 750 mg bd (n=5) DL2 NFV1250 mg bd (n=3) DL3 NFV 100 mg bd (n=3)	DLT pCR TRG	G3 transaminase (2) G3 cholangitis (1) G3 ileus G3 diarrhea (2) G4 post-op wound complication (1)	G3 diarrhea (2) at DL2 G3 transaminase (2) G3 cholangitis (1) G3 ileus G4 post-op wound complication (1) At DL3	pCR 3/11 (27%) Good TRG 4/11
Alonso – Basanta et al. (33)	Glioblastoma (post-op)	21	NFV 7-10 days before and concurrent with: 60 Gy in 30# GTV and Temozolomide 75 mg/m2 od DL1 NFV 625 mg bd (n=3) DL2 NFV 1250 mg bd (n=18)	DLT PFS OS	Diarrhea (1) Transaminase (8) Bilirubin (1) Alkaline phosphatase (1) Lymphopenia (2)	G3 hepatotoxicity (3) G3 diarrhea (1) at DL2	Median PFS 7.2 months Median OS 13.7 months

Abbreviations used: CR, Complete Response; CT, Computed Tomography; DL, dose level; DLT, Dose Limiting Toxicity; G3/4, Grade 3/4; NFV, Nelfinavir; OS, Overall Survival; pCR, pathologic Complete Response; PFS, Progression Free Survival; PR, Partial Response; PET, Positron Emission Tomography; SD, Stable Disease; TRG, Tumour Regression Grade;

Table 2: Clinical and Radiological Patient Characteristics at Baseline

Characteristic	SONATINA patients (N=10) No.	%
Age(years)		
Median	65	
Range	45-81	
Gender		
Male	5	50
Female	5	50
ECOG Performance Status		
0	4	40
1	6	60
Sub-site of tumor in rectum		
Low	7	70
Mid	2	20
Upper	1	10
MRI defined T-stage		
T3	4	40
T4	6	60
MRI defined N-stage		
N0	2	20
N1	3	30
N2	5	50
Sites of metastatic disease (CT)		
Liver	8	
Distant lymph nodes	5	
Lung	6	
Other	1	

Abbreviations used: MRI, magnetic resonance imaging; CT, computed tomography.

Table 3: Toxicities observed up to 28 days from the last fraction of RT			
	No. of toxicities		
Toxicity	**CTCAE Grade 0-2**	**CTCAE Grade 3** {Nelfinavir causality}	**CTCAE Grade 4**
Anemia	1 (1 patient)	0	0
Anorexia	2 (2 patients)	0	0
Diarrhea	7 (6 patients)	2 (2 patients) {probably related, definitely not related}	0
Fatigue	8 (7 patients)	0	0
Fever	1 (1 patient)	0	0
Gastrointestinal – other	7 (5 patients)	0	0
Hyperglycemia (fasting glucose)	3 (3 patients)	0	0
Hyponatremia	0	1 (1 patient) {probably not related}	0
Lymphopenia	2 (2 patients)	2 (1 patient) {possibly related, definitely not related}	0
Nausea/vomiting	12 (5 patients)	1 (1 patient) {possibly related}	0
Other	8 (7 patients)	0	0
Pain	3 (3 patients)	0	0
Peripheral neuropathy	2 (2 patients)		
Proctitis/perianal pain	3 (3 patients)	1 (1 patient) {probably not related}	0
Rash	4 (4 patients)	1 (1 patient) {probably related}	0
Urinary symptoms	5 (3 patients)	0	0
Total	**68**	**8** (7 patients)	**0**

Table 4: Tumour response on MRI 8 weeks post therapy (mrTRG score) for individual patients in relation to baseline characteristics and number of cycles of chemotherapy administered.

Patient number	Baseline MRI stage	*KRAS* mutation status	HIF1-alpha expression at baseline	CAIX expression at baseline	Phospho-PRAS40 Expression at baseline	No. weeks of oxaliplatin-fluouracil chemotherapy between end of RT and MRI	mrTRG score
1	T3b N2	Wild-type	negative	positive	negative	6	good
2	T4 N2	Mutant (G12V)	negative	negative	negative	6	poor
3	T3a N2	Wild-type	positive	negative	positive	6	good
4	T3b N2	Mutant (G12A)	Not evaluable	positive	negative	6	poor
5	T3a N2	Mutant (G12S)	positive	negative	negative	3	good
6	T4 N2	Mutant (G12V)	negative	negative	negative	3	poor
7	T4 N2	Wild-type	negative	positive	positive	4	poor
8	T4 N2	Mutant (G12V)	negative	Not evaluable	Not evaluable	4	good
9	T4 N1	Mutant (G12C)	positive	positive	positive	None	good
10	T4 N2	Mutant (G13A)	positive	negative	negative	None	N/A

Abbreviations used: mrTRG, Tumour Regression Grade on MRI 8 weeks post radiotherapy; Ox/MDG, Oxaliplatin and Modified de Gramont; CAPOX, Capecitabine and Oxaliplatin