piccolo

Panitumumab, **I**rinotecan & **C**iclosporin
in **COLO**rectal cancer therapy

A randomised clinical trial of treatment for fluorouracil-resistant advanced colorectal cancer

comparing
standard single-agent irinotecan
versus
irinotecan plus panitumumab*
and versus
irinotecan plus ciclosporin**

*(in patients with *K-RAS* wild-type tumours)
**(in patients with *K-RAS* mutated or undefined tumours)

Developed by the UK National Cancer Research Institute (NCRI)
Colorectal Clinical Studies Group,
Advanced Disease and Translational Research Subgroups

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FOREWORD TO PROTOCOL VERSION 3.0

PICCOLO Protocol Version 3.0 is a substantial amendment from previous versions, incorporating prospective *K-RAS* gene testing, with different randomisations for patients with tumours of differing *K-RAS* mutation status. Investigators are therefore advised to read this protocol carefully, and to ensure that copies of previous versions of the protocol are removed from clinical areas.

Patients already randomised under the previous versions of PICCOLO should continue their treatment using this new protocol, since the treatment schedules, dose adjustments, and all other details of treatment after randomisation, remain the same.

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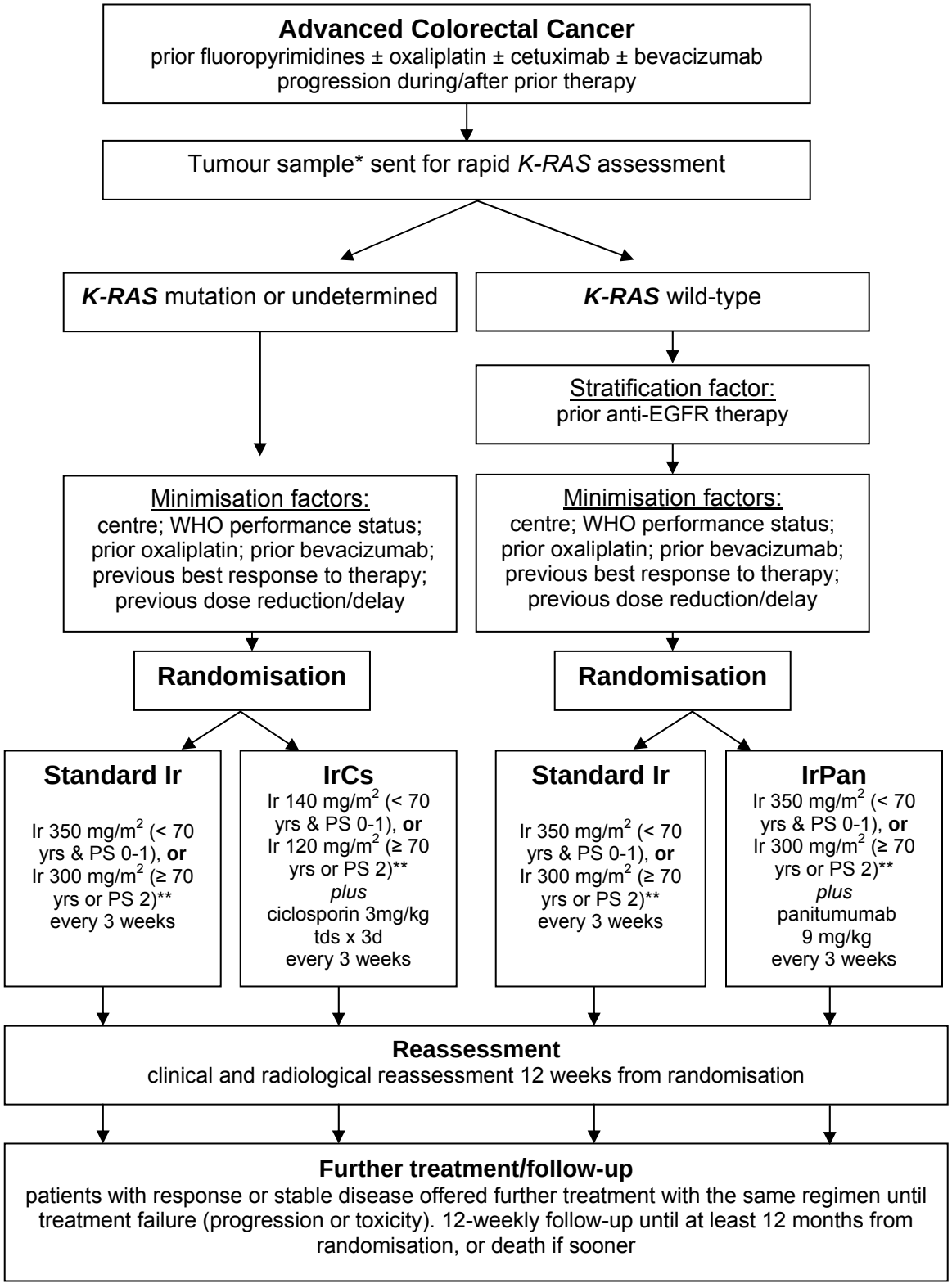
PICCOLO Flow Diagram



** Formalin-fixed paraffin-embedded blocks from primary and/or metastatic tumour. See section 5.1*
*** The lower dose of irinotecan is for patients aged ≥70 years, or of any age if WHO performance status 2. If the lower dose is well tolerated for the first two cycles it may be escalated to the full dose thereafter.*

GLOSSARY OF TERMS

AE	Adverse Event
AUC	Area under the plasma concentration-versus-time curve
CR	Complete Response
CRF	Case Report Form
Cs	Ciclosporin
CTA	Clinical Trials Authorisation
CTC	Common Toxicity Criteria
CTRU	Clinical Trials Research Unit
DMEC	Data Monitoring and Ethics Committee
DLQI	Dermatology Life Quality Index
EGFr	Epidermal Growth Factor Receptor
EQ-5D	EuroQol Questionnaire
GCP	Good Clinical Practice
Ir	Irinotecan
IrCs	Irinotecan plus ciclosporin
IrPan	Irinotecan plus panitumumab
LFTs	Liver Function Tests
LREC	Local Research Ethics Committee
MHRA	Medicine and Healthcare Products Regulatory Agency
NCI	National Cancer Institute
NICE	National Institute of Health and Clinical Effectiveness
OS	Overall Survival
PA	Patient Acceptability
Pan	Panitumumab
PD	Progressive Disease
PFS	Progression-free Survival
PK	Pharmacokinetics
PR	Partial Response
QL	Quality of Life
EORTC QLQ-C30	European Organisation for Research & Treatment of Cancer questionnaire
REC	Research Ethics Committee
SAE	Serious Adverse Event
SD	Stable disease
SNP	Single Nucleotide Polymorphism
SUSARs	Suspected Unexpected Serious Adverse Reactions
U&Es	Urea & Electrolytes
VEGF	Vascular Endothelial Growth Factor
WBC	White Blood Cells
WHO	World Health Organisation

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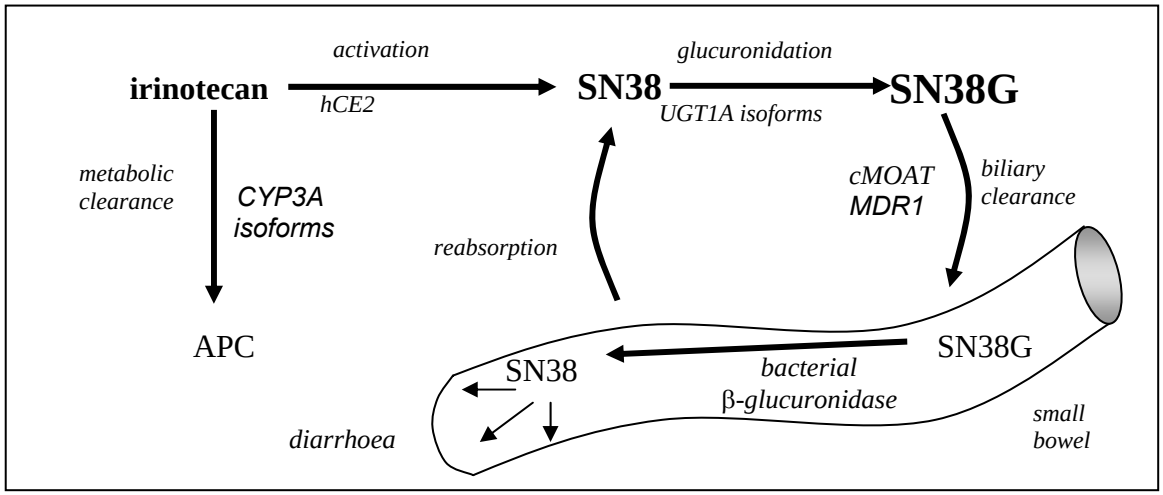
1. BACKGROUND

The management of colorectal cancer has moved on rapidly in recent years with the introduction of several new active agents. Under current NICE guidance, most metastatic colorectal cancer patients in the UK are treated initially with fluoropyrimidine (FP) therapy or the combination of FP plus oxaliplatin. However for nearly all these patients, resistance to 1st-line therapy eventually develops. At that point NICE recommends consideration of 2nd-line therapy with single-agent irinotecan (Ir), an inhibitor of topoisomerase-I which shows little cross-resistance with FPs and oxaliplatin. As well as its role in metastatic colorectal cancer, Ir is also being evaluated in the adjuvant setting, and in a range of other tumour types.

Irinotecan's chief toxicity of concern is delayed diarrhoea, which may be severe and, when combined with myelosuppression, even life-threatening. In the NCRI/MRC FOCUS trial, patients receiving standard single-agent irinotecan had a 17% risk of experiencing CTC grade ≥3 diarrhoea (defined as an increase of ≥7 stools per day, incontinence, or the need for parenteral rehydration)[1] An independent review of two large randomised trials highlighted an unacceptable rate of toxic deaths in patients receiving Ir with bolus 5FU.[2] Aggressive management with loperamide and antibiotics may limit this risk, but prevention of Ir-induced diarrhoea would be preferable.

Irinotecan and ciclosporin

The pharmacokinetics (PK) and toxicity of Ir depend partly on genetic factors.[3,4] It is a prodrug, converted by carboxylesterases to the active SN38. There is a metabolic inactivation pathway mediated by CYP3A4, but more important is the glucuronidation and biliary excretion of SN38. Hepatic glucuronidation to SN38G is by UGT1A1, and canalicular transport of both SN38 and SN38G into the biliary tract is mediated by cMOAT (ABCC2; MRP2) and MDR1 (P-glycoprotein).[5,6] In the bowel, bacterial β-glucuronidase can convert SN38G back to SN38 which is reabsorbed, resulting in an enterohepatic recirculation loop. This leads to a high concentration of SN38 in the small bowel[7] and is thought to be responsible for the late diarrhoea.[8,9]



Correlations have been reported between a single nucleotide polymorphism (SNP) in UGT1A1 and the toxicity and activity of Ir.[10,11] SNPs have also been identified in other relevant enzymes including the UGTs 1A6, 1A7 and 1A9, and CYP3A4, CYP3A5, in some cases with early suggestions of an association with Ir toxicity or activity.[12]

Ciclosposin (Cs) is a cyclical oligopeptide which induces reversible suppression of T-lymphocyte function, and is used primarily as a suppressor of allotransplant rejection. It does not suppress haematopoiesis or granulocyte function. Its oral bioavailability is improved and made more consistent using a formulation which undergoes microemulsification in water (Neoral™), available as soft gelatine capsules and as liquid.[13]

Cs is extensively metabolised, and is excreted in bile. It inhibits both cMOAT and MDR1 and, thereby, the biliary excretion of SN38G.[14,15] A Chicago group reported a study in 53 patients given intravenous infusion Cs alongside weekly Ir. PK analysis confirmed reduced clearance and increased half-life of Ir compared with historical controls treated with Ir alone.[16] Following that finding, a trial was performed in 37 patients in Leeds, giving oral Cs for 3 days every 2 weeks, with Ir given on the second day. Dose escalation established the optimum dose of Ir with Cs, and PK analysis of Ir, SN38 and SN38G was performed in 20 patients during paired cycles of Ir alone and IrCs.[17] Cs reduced the clearance of Ir, SN38 and SN38G, and increased their AUCs, by factors of around 2.3-fold. There was a proportionate reduction in the optimum dose of Ir to 100 mg/m^2 fortnightly, compared with the standard dose of 250 mg/m^2 fortnightly. Most importantly, only one of the 37 patients in the study had grade ≥3 diarrhoea compared with, typically, 20% of patients treated with standard regimens of Ir alone. Systemic side-effects (myelosuppression; alopecia) and anticancer activity (response rate; PFS) were similar to standard single-agent Ir.[17]

As an extension to the Leeds trial, a further 35 patients received IrCs at the recommended doses of 100 mg/m^2 irinotecan and 5mg/kg ciclosporin b.d. for three days, on a fortnightly cycle. A median of 5 cycles per patient were given. Again, the regimen was well tolerated, the principle side-effects being neutropenia and nausea/vomiting (5 patients (15%) each of grade >2) and alopecia. Only one patient (3%) experienced Grade 3 diarrhoea.[18]

Continuous dosing with Cs, for example in transplant patients, is associated with a wide range of unwanted effects including renal impairment, hypomagnesaemia, neurological and gastro-intestinal symptoms. The three-days fortnightly pulses of Cs used in the initial Leeds IrCs study were tolerated with minimal side-effects, and no renal toxicity occurred. However, in the extension study, 6/35 (18%) patients required reduction or cessation of ciclosporin for side-effects, most commonly nausea or abdominal cramps, and after discussion with transplant physicians a schedule adjustment has been made to give ciclosporin at 3 mg/kg t.d.s.

Panitumumab

Of the novel colorectal cancer treatment approaches currently being clinically evaluated, the most promising are those targeting VEGF and EGFr. EGFr is a transmembrane glycoprotein which, in response to binding of ligand (such as EGF or TGFα) to its extracellular domain, generates intracellular tyrosine kinase activity, stimulating an intra-cellular cascade leading to cell cycle progression and other cellular events important in carcinogenesis. EGFr expression, as detected by immunohistochemistry is seen in around 70% of colorectal cancer cells. Two approaches to therapeutic inhibition of EGFr tyrosine kinase activity are currently under evaluation: monoclonal antibodies (mAbs) to block the ligand-binding domain (e.g. cetuximab; panitumumab), and small molecule inhibitors of the intracellular tyrosine kinase (e.g.: gefitinib; erlotinib).

In colorectal cancer, the more successful of these to date has been the mAb approach, with the compound cetuximab already licensed for use in patients with irinotecan-resistant colorectal cancer, following a series of phase II clinical trials. These trials demonstrate that around 10% of patients with disease progressing on or soon after fluoropyrimidine and irinotecan therapy will have a RECIST partial response to single-agent cetuximab, and around 20% will have a

response to cetuximab + irinotecan.[19,20] The higher response rate with the combination, even in patients with irinotecan-insensitive disease, tends to support the *in vitro* suggestions of additivity between these two agents, with EGFr inhibition stimulating responsiveness to irinotecan or *vice versa*.

Panitumumab is a high affinity (K_d = 5 x10^{-11}M) IgG_2 mAb directed against the extracellular domain of EGFr. It differs from cetuximab in being fully human, having been generated in transgenic animals in which the murine heavy and light chain loci have been inactivated and most of the human heavy and light chain (both λ and κ) Ig loci inserted. It blocks the binding of ligands to EGFr in multiple human tumour cell lines and inhibits EGFr tyrosine phosphorylation, *in vitro* cell proliferation and xenograft tumour growth in a dose-dependent manner.[21] There is only a partial relationship between the number of EGFr molecules per cell and xenograft growth inhibition.[22]

In a large phase II trial,[23] 148 patients with colorectal cancer resistant to 2 or more chemotherapy drugs, and with at least weak tumour EGFr expression by immunohistochemistry, received single-agent panitumumab at 2.5 mg/kg weekly. Partial responses were seen in 9%, with stable disease in a further 29% - similar results to those seen with single-agent cetuximab.[19] The median duration of response was 18.1 weeks (95% C.I.: 17.9, 23.3), median progression free survival was 13.6 weeks (8.3, 16.1), and median overall survival was 37.6 weeks (25.6, 42.4). As previously reported with cetuximab,[19] no relationship was found between the intensity of EGFr staining and response to panitumumab.

As with cetuximab and other EGFr inhibitors, the most commonly reported side effects of panitumumab are dermatological. In a phase II trial in pretreated metastatic colorectal cancer, skin toxicity was reported by almost all subjects (95%), and reached NCTC Grade 3 in 7%.[23] Also in common with cetuximab, hypomagnesaemia has been reported in some trials. Routine monitoring was not conducted in the early clinical studies, so the incidence of asymptomatic hypomagnesaemia is not yet clear. However, grade 3 or 4 hypomagnesaemia has been reported through serious adverse event notifications in 7 of 1100 (0.6%) subjects receiving panitumumab to date. Overall the most commonly reported treatment-related side effects are fatigue (28%), diarrhoea (22%) nausea (16%), stomatitis (14%), anorexia (12%) and vomiting (7%). Grade 3 events were reported for fatigue (3%), diarrhoea, nausea and vomiting (reported at 1% each). Intensive cardiac monitoring was undertaken in the early clinical trials with panitumumab, including MUGA and cardiac enzyme monitoring in 331 patients, but no evidence of cardiotoxicity was observed (see table 1).[24]

From the start of panitumumab clinical studies to 19 June 2005, 1575 patients were exposed. From 20 June 2004 to 19 June 2005, 4 related hypersensitivity events (SUSARs) were reported. Routine pre-medication is not recommended, but intervention is required when clinically indicated (e.g. i.v. steroids; antihistamines; adrenaline; oxygen, etc).

Table 1 AE's observed in a trial of monotherapy in mCRC (failed irinotecan +/- oxaliplatin):

All Patients (N = 148) **Skin related**	All AES	Grade 3	Grade 4
Any Skin Toxicity	141 (95%)	11 (7%)	0 (0%)
Rash	119 (80%)	5 (3%)	0 (0%)
Pruritus	50 (34%)	2 (1%)	0 (0%)
Dry skinDermatitis	38 (26%)	0 (0%)	0 (0%)
acneiform	24 (16%)	0 (0%)	0 (0%)
Skin desquamation	19 (13%)	0 (0%)	0 (0%)
Skin disorder	18 (12%)	0 (0%)	0 (0%)
Erythema	16 (11%)	0 (0%)	0 (0%)
Paronychia	16 (11%)	0 (0%)	0 (0%)
Rash macular	15 (10%)	0 (0%)	0 (0%)
Skin fissures	14 (9%)	1 (1%)	0 (0%)
Rash maculo-papular	11 (7%)	0 (0%)	0 (0%)
Rash papular	10 (7%)	0 (0%)	0 (0%)
Rash pruritic	9 (6%)	0 (0%)	0 (0%)
Dermatitis exfoliative	8 (5%)	0 (0%)	0 (0%)
Other			
Fatigue	41 (28%)	4 (3%)	0 (0%)
Diarrhoea	32 (22%)	1 (1%)	0 (0%)
Nausea	23 (16%)	1 (1%)	0 (0%)
Stomatitis	21 (14%)	0 (0%)	0 (0%)
Anorexia	18 (12%)	0 (0%)	0 (0%)
Vomiting	10 (7%)	2 (1%)	0 (0%)

Panitumumab has non-linear pharmacokinetics in humans. With repeated dosing (and saturation of EGFr) at the dose of 2.5 mg/kg its clearance rate of <5 mL/day/kg is close to that of endogenous IgG_2, and interpatient variability is relatively low. This makes feasible an infrequent administration schedule. PK data for the 9 mg/kg 3-weekly schedule used in the PICCOLO protocol are available for 22 patients (15 treated with CHO-derived protein; 7 with an earlier formulation of hybridoma-derived protein) and are summarised here:[24]

Mean (sd) values	After 1st dose		After 3rd dose	
	CHO (n=16)	Hybridoma (n=5)	CHO (n=6)	Hybridoma (n=2)
$AUC_{0\text{-}tau}$ (ug.day/mL)	1597 (274)	1801 (717)	2180 (415)	2593 (252)
C_{max} (ug/mL)	226 (51)	253 (80)	245 (40)	292 (15)
CL (mL/day/kg)	5.24 (1.29)*	5.27 (2.44)	4.27 (0.92)	3.49 (0.34)
T½ (day)	7.40 (2.37)	5.91 (2.02)	8.31 (1.31)	8.82 (1.08)

*n=12

Prior to PICCOLO, no trials have combined panitumumab with single-agent irinotecan, but in an ongoing phase II trial panitumumab 2.5 mg/kg once weekly is added to 1st- line irinotecan, 5FU and leucovorin. Initially, 19 patients were treated using the IFL "Saltz Regimen" but, in common with other trials using that regimen, unacceptable levels of toxicity were seen, with diarrhoea (any grade) in 89% patients, and 58% patients experiencing SAEs. A further 17 patients have now been recruited using panitumumab plus the less toxic FOLFIRI regimen, and preliminary safety data are satisfactory.[25] That trial includes a pharmacokinetic sub-study, which shows that IFL does not affect the PK profile of panitumumab and, conversely, panitumumab does not affect the pharmacokinetics of irinotecan or SN38.

Rationale for PICCOLO

Thus, two potential modifications to standard irinotecan therapy are proposed. Firstly, pharmacokinetic modulation of irinotecan with ciclosporin has the potential to deliver equally efficacious treatment but avoid its principal toxicity, diarrhoea. Secondly, the addition of EGFr inhibition in the form of panitumumab has the potential to increase the efficacy of treatment whilst maintaining a convenient 3-weekly administration schedule.

PICCOLO is a large randomised trial designed to address both of these modifications, while at the same time acquiring translational scientific information to further refine and direct these treatments in the future. A common control arm of standard single-agent irinotecan will be compared, on the one hand, with ciclosporin-modulated irinotecan and, on the other, with irinotecan plus panitumumab.

The large majority of patients entering PICCOLO will not have received a prior EGFr-inhibiting drug. However, the trial is open to patients following first-line treatment in COIN, one-third of whom will have received cetuximab. These anti-EGFR targeted therapy pre-treated patients will not be included in the primary +/- panitumumab phase III efficacy comparison, but will provide the opportunity to explore, in a randomised phase II analysis, the possibility that EGFr blockade contributes to the efficacy of second-line chemotherapy independent of its use during first-line treatment.

On this basis, PICCOLO opened to recruitment in December 2006 as a 3-arm Randomised Controlled Trial (RCT), with all patients randomised, in equal proportion, to the three study arms **Ir** (standard single-agent irinotecan), **IrCs** (irinotecan at 40% standard dose in combination with ciclosporin), and **IrPan** (standard irinotecan in combination with panitumumab).

K-RAS Mutations and anti-EGFR mAb therapy (section added for Protocol Version 3.0)

Following initiation of the PICCOLO Trial as a 3-arm randomised trial, new data have emerged to suggest that patients can benefit from the application of a molecular selection strategy. This new data necessitates a modification of the original PICCOLO trial design.

K-RAS, the human homolog of the Kirsten rat sarcoma-2 virus oncogene, encodes a GTP-binding protein that acts as a self-inactivating signal transducer by cycling from GDP- to GTP-bound states in response to stimulation of cell surface receptors including EGFR.[26,27] Certain codons in *K-RAS* (12, 13 and 61) are common sites of mutations that yield constitutively active protein.[28,29] Such mutations are found in approximately 30-50% of colorectal cancers.[30] The presence of mutant *K-RAS* in lung and colorectal cancers is a poor prognostic factor,[30,31] and several reports from non-randomised series suggested association with lack of response to EGFR inhibitors.[32,33]

In April 2008, published data from a large RCT showed convincingly that patients with *K-RAS* wild-type tumours gained prolongation of progression-free survival if randomised to receive panitumumab, but patients whose tumours had mutated *K-RAS* gained no benefit.[34] This interaction was significant at $p<0.0001$, suggesting that *K-RAS* is a potentially valuable predictive biomarker for panitumumab. These findings have now been validated by data from three further RCTs showing that whilst patients with wild-type *K-RAS* tumours may benefit from either panitumumab or cetuximab, patients with mutated *K-RAS* tumours do not.[35,36,37] In *KRAS*-mutation patients receiving oxaliplatin +/- bevacizumab, addition of an anti-EGFR drug was observed to shorten the time to progression, suggesting a possible antagonistic interaction[35,37] but this was not seen in trials of anti-EGFR drugs alone or in combination with irinotecan.[34,36]

Given this new information, the PICCOLO Trial Management Group, in consultation with the NCRI Colorectal Clinical Studies Group and independent Trial Steering Committee, decided to modify the PICCOLO trial design from mid-2008. This decision was made for two reasons:

- With the weight and consensus of evidence now suggesting that patients with *K-RAS*-mutated tumours cannot benefit from anti-EGFR mAb, it is important to avoid patients in this group being exposed to panitumumab and risking its adverse effects with no realistic prospect of benefit.
- The future development of anti-EGFR monoclonal antibody therapy in colorectal cancer will be restricted to patients with demonstrable *K-RAS* wild-type tumours. Therefore, the principal question addressed by the PICCOLO trial, whether the addition of panitumumab to irinotecan improves survival in patients with colorectal cancer, must now be up-dated to whether the addition of panitumumab to irinotecan improves survival *in patients with K-RAS-wild-type* colorectal cancer.

In the new design, patients undergoing screening for inclusion in PICCOLO will be asked for consent to retrieve a sample of tumour (usually formalin-fixed, paraffin-embedded blocks from a previous primary resection or metastatic biopsy procedure). This sample will be encoded and sent to a reference laboratory for *K-RAS* mutation assessment using an approved assay technique. The result of the assay will then be communicated directly to the Clinical Trials Research Unit (CTRU). Subsequent randomisation will be as shown in the trial flow diagram on page 5.

2. AIMS AND OBJECTIVES

PICCOLO has two separate clinical objectives. These are:

Objective 1 (irinotecan modulation with ciclosporin): to establish whether the toxicity of irinotecan therapy is reduced, without loss of efficacy, by modulation with ciclosporin

Objective 2 (addition of panitumumab): to establish whether, in patients with *K-RAS* wild-type tumours, the efficacy of irinotecan therapy is improved by the addition of panitumumab

To address these objectives, the trial poses specific questions using primary and secondary endpoints, fully described in section 10. The general approach is:

Objective 1 (comparison: **Ir** *versus* **IrCs**).

- A sensitive primary outcome measure – proportion of patients alive without evidence of disease progression 12 weeks after randomisation – is used to establish non-inferior anti-cancer efficacy for the **IrCs** combination

- Provided this non-inferiority condition is satisfied, secondary outcome measures will be used to determine whether **IrCs** has improved tolerability (reduced diarrhoea; reduced overall toxicity; improved patient acceptability and/or improved quality of life). In addition, overall survival will be used as a secondary outcome measure to confirm non-inferiority.

Objective 2 (comparison: **Ir** *versus* **IrPan**).

- A robust primary outcome measure – overall survival – is used to establish whether, in patients with *K-RAS* wild-type tumours not previously treated with anti-EGFr therapy, **IrPan** has superior efficacy to Ir.

- Secondary outcome measures will be used to compare the toxicity, patient acceptability and quality of life associated with the two regimens in patients not previously treated with anti-EGFr therapy. Secondary anti-cancer efficacy measures of disease response and progression will also be applied to assess superiority of IrPan in these patients.

- A small proportion of patients will have received a prior anti-EGFR targeted therapy. They will be considered in a separate exploratory comparison of efficacy using a sensitive outcome measure (proportion of patients progression-free at 12 weeks) to address the question whether panitumumab enhances irinotecan sensitivity in this context and to also assess toxicity. This exploratory analysis will consider only those patients with wild-type *K-RAS* status who have previously received an anti-EGFR targeted therapy.

- There will also be some patients randomised under the Protocol Version 1.0 who have received IrPan and whose *K-RAS* status is determined retrospectively to be mutant or unknown. These patients will be considered in a separate exploratory comparison of efficacy using a sensitive outcome measure (proportion of patients progression-free at 12 weeks) to address the question whether panitumumab impedes irinotecan sensitivity in this context and also to assess toxicity. This exploratory analysis will consider all

patients randomised to receive Ir or IrPan under Protocol Version 1.0 whose *K-RAS* status is unknown or mutant, regardless of previous anti-EGFR targeted therapy use.

Translational objectives

PICCOLO also addresses the following translational research objectives

- Does the toxicity of **Ir** and/or **IrCs** correlate with genetic variability in the enzymes involved in irinotecan's disposition pathway?
- Does **IrCs** achieve the same PK exposure (AUC) to Ir and its metabolites (SN38; SN38G) as standard-dose **Ir**? (This study in a subset of 20 patients)
- If, overall, **Pan** is effective in patients with *K-RAS* wild-type tumours, does the degree of benefit correlate with other molecular markers which may provide a basis for more refined patient selection in the future (e.g. alterations in EGFr; *B-RAF, MAPK, STAT, P13K*, etc)?
- Does **Pan** efficacy or toxicity (specifically the severity of skin rash) correlate with somatic alterations in the EGFr gene, or with germline variability in related genes?

3. TRIAL DESIGN

PICCOLO is a multi-centre, open-label, randomised, controlled, 4 arm clinical trial designed to show whether, regardless of tumour subtype, the novel regimen **IrCs**, is non-inferior for anti-cancer efficacy and superior for toxicity compared to **Ir**, and whether, in patients with *K-RAS* wild-type tumours, the addition of panitumumab to irinotecan, **IrPan**, gives superior anti-cancer efficacy compared to **Ir** alone.

Potential PICCOLO patients are invited to consent to retrieval of stored tumour samples to test for *K-RAS* mutations at codons 12, 13 and 61. Thereafter, patients who fulfil the eligibility criteria and give informed consent are randomised according to their tumour *K-RAS* status as shown in the flow diagram on page 5.

Patients whose tumours are shown to have a mutation in *K-RAS*, and those in whom *K-RAS* genotyping is not possible due to assay failure or unavailability of tissue, are allocated on a 1:1 random basis to receive **Ir** or **IrCs**. This randomisation uses the minimisation factors listed in the flow diagram.

Patients whose tumours are wild-type for *K-RAS* are allocated on a 1:1 random basis to receive **Ir** or **IrPan**. This randomisation is stratified according to whether or not the patient has received prior anti-EGFR targeted therapy (e.g. cetuximab or panitumumab), and uses the minimisation factors listed in the flow diagram.

Recruitment to PICCOLO will continue until 480 non-EGFR-pre-treated *K-RAS* wild-type patients have entered the **Ir** vs. **IrPan** randomisation (240 patients per arm), and 750 patients have entered the Ir vs. IrCs randomisation (375 patients per arm). The Ir vs. IrPan comparison includes approximately 143 patients fulfilling these criteria who were randomly allocated to **Ir** or **IrPan** under Protocol Version 1 (in whom *K-RAS* status is being determined retrospectively), plus approximately 337 patients to be recruited using Protocol Version 2. The Ir vs. IrCs comparison includes 329 patients randomly allocated to **Ir** or **IrCs** under Protocol Version 1 (regardless of their *K-RAS* status), plus approximately 421 patients to be recruited using Protocol Version 2. The recruitment ratio to the Ir vs. IrCs comparison and the Ir vs. IrPan comparison is dependent upon the proportion of patients with unknown *K-RAS* status (which we

anticipate to be around 15%). This will be monitored throughout the study as this may vary. In order to achieve the required number of patients for each comparison we will recruit approximately 1324 patients in total (494 already recruited under Protocol Version 1; 830 using Protocol Version 2, assuming 15% of patients with unknown *K-RAS* status, and 15% of patients have previously received anti-EGFR targeted therapy).

Patients randomised using the previous version of the protocol, without advance knowledge of *K-RAS* status, will have their *K-RAS* status determined retrospectively (provided consent has been obtained for tissue retrieval), and will be incorporated in the relevant treatment comparisons as detailed in Section 11.

In addition, approximately 86 anti-EGFR targeted therapy pre-treated patients who have wild-type *K-RAS* status will be available for an assessment of the efficacy of **Ir** versus **IrPan** in this pre-treated group. Similarly, patients randomised to Ir or IrPan using the previous version of the protocol, who are retrospectively found to have mutant or unknown K-RAS status, will be available for an assessment of the efficacy of **Ir** versus **IrPan** in this group of patients

All PICCOLO patients are studied for clinical and pharmacogenetic endpoints. All patients are also invited to donate any surplus stored formalin-fixed paraffin-embedded (FFPE) tumour samples for translational research. A pharmacokinetic (PK) Sub-study is being undertaken in 20 patients randomised to IrCs at centres with appropriate facilities in order to compare Ir, SN38 and SN38G pharmacokinetics after Ir or IrCs treatment.

4. ELIGIBILITY

The eligibility criteria are designed to include, as far as possible, any patient for whom standard single-agent irinotecan would normally be a treatment option, in accordance with its marketing licence and current NICE guidance.

Patient Selection

The Cancer Diagnosis

- Confirmed advanced colorectal adenocarcinoma:
    - **either** previous or current histologically confirmed primary adenocarcinoma of colon or rectum, together with clinical/radiological evidence of advanced/ metastatic disease
    - **or** histologically/cytologically confirmed metastatic adenocarcinoma, together with clinical/radiological evidence of colorectal primary tumour
- Unidimensionally measurable disease (RECIST criteria, see Appendix 1)
- Not known to have CNS metastases or carcinomatous meningitis

Previous Anticancer Treatment

- Patient must have had prior fluoropyrimidine +/- oxaliplatin therapy, with disease progression during or after that treatment:
    - adjuvant therapy and/or prior therapy for advanced disease may have been given.
    - if disease has progressed after but not during prior therapy, the treating consultant should be satisfied that further fluoropyrimidine (+/- oxaliplatin) therapy is not appropriate.

  - if the only prior chemotherapy was given adjuvantly, progression should have occurred during or <3 months after completing the adjuvant course, and the treating consultant should be satisfied that further fluoropyrimidine (+/- oxaliplatin) therapy is not appropriate
- No previous treatment with irinotecan may have been given
- Prior anti-EGFR therapy (cetuximab; panitumumab) and/or bevacizumab is acceptable, but patients with a prior anaphylactic reaction to cetuximab should not be entered.
- Please note the minimum acceptable time intervals from the end of previous therapy to the planned start of PICCOLO treatment:
  - Capecitabine: 14 days
  - All other licensed cytotoxic drugs: 21 days
  - Prior cetuximab, panitumumab or bevacizumab: 21 days
  - Any experimental anticancer drug therapy including antibodies: 42 days

  Note also that PICCOLO treatment must start as soon as possible (maximum 14 days) after randomisation.
- Prior radical pelvic radiotherapy is not an absolute contraindication, however patients with ongoing radiotherapy toxicity, especially diarrhoea, should not be entered into PICCOLO.

Patient Fitness and Co-morbidity

- WHO performance status of 0, 1 or 2, with estimated life expectancy of at least 12 weeks
- Full blood count:
  - Hb ≥10.0 g/dl; WBC ≥3.0 $x10^9$/l; Plts ≥100 $x10^9$/l
- Renal biochemistry:
  - GFR calculated by the Cockcroft formula ≥50 ml/min, or measured GFR (EDTA or 24hr creatinine clearance) ≥60ml/min.
- Hepatobiliary function:
  - ALP ≤ 5 x Upper Limit of Normal (ULN), and AST or ALT ≤ 2.5 x ULN
  - bilirubin ≤ 25 umol/l, and no clinical or radiological evidence of biliary obstruction
  - no known history of Gilbert's syndrome
- Medical comorbidity:
  - no ongoing requirement for for ciclosporin or a contraindicated concomitant medication (see section 6.6). Short-course antibiotics or antifungals are acceptable if they will finish > 5 days before start of PICCOLO therapy.
  - no concurrent or previous other cancer that could confuse diagnosis (non-melanomatous skin cancer or superficial bladder cancer acceptable; for other cases please discuss with CTRU)
  - no other serious medical condition: e.g.: major surgery within preceding four weeks, unresolved bowel obstruction; uncontrolled infection, uncontrolled chronic enteropathy (e.g. Crohn's disease, ulcerative colitis), or chronic diarrhoea (≥4 stools per day) of any cause
  - no clinical or radiological evidence of interstitial pneumonitis, pulmonary fibrosis, pleural effusion or ascites causing grade ≥2 dyspnea

Other Factors

- Aged ≥18 years
- Capable of oral self-medication, reporting toxicity and completing QoL questionnaires
- If female and of childbearing potential, must:
  - have a negative pregnancy test within 72 hours prior to trial entry, and not be breastfeeding

  - agree to use adequate, medically approved, contraceptive precautions (oral or barrier contraceptive under the supervision of a General Practitioner or Family Planning Clinic) during and for 6 months after study treatment
- If male with a partner of childbearing potential, must:
  - agree to use adequate, medically approved, contraceptive precautions (oral or barrier contraceptive under the supervision of a General Practitioner or Family Planning Clinic) during and for 6 months after study treatment

5. REGISTRATION AND RANDOMISATION

Direct line for 24-hour registration & randomisation: 0113 343 7957

Recruitment to PICCOLO is a 2-step process:

1. Registration: an initial introduction to the trial is given and written assent for *K-RAS* testing is obtained. A PICCOLO trial ID number will be assigned to the patient at this stage.
2. Randomisation: a full explanation of the trial including detailed information about the rationale, design and personal implications of the study is given and formal consent to randomisation is obtained. At this stage the treatment allocation will be determined.

The minimum interval between these stages is 24 hours, but this will apply only to patients whose *K-RAS* status has been previously determined. Most patients will require *K-RAS* testing during this time, so the normal interval will be 1-2 weeks.

A record of the consent processes detailing the date of consent and all those present will be kept in the patient notes. Both the *K-RAS* Testing and PICCOLO Consent Forms must be signed by an authorised medically qualified person. The right of the patient to refuse consent without giving reasons will be respected. Further, the patient will remain free to withdraw from the study at any time without giving reasons and without prejudicing any further treatment. The original consent forms should be filed in the Investigator Site File, a copy should be given to the patient and a further copy of the consent form should be sent to the Clinical Trials Research Unit (CTRU) at the University of Leeds. A copy of the Patient Information Sheet and Consent Form document should be filed in the patient hospital notes if required.

Participating centres will register and randomise patients using the 24-hour automated registration/randomisation system based at the CTRU. A centre code, authorisation code and PIN, which will be provided by the CTRU, will be required to access this system. These codes will only be issued once a centre has been fully approved and all the necessary documentation has been received. Precise details of documentation required prior to the start of recruitment at each centre will be outlined on expression of interest from participating centres.

5.1 Registration

When a patient is identified who may potentially be suitable for PICCOLO a verbal introduction to the trial is given and the *K-RAS* Testing Patient Information Sheet/Consent Form is provided and sufficient time allowed for the patient to consider it. In some cases, the clinician or research nurse may feel it more appropriate to arrange an extra visit to obtain written consent for *K-RAS* testing. It is made clear to the patient that this is not consent to participate in the PICCOLO trial:

they remain free to decide not to participate, or they may be found to be ineligible after the formal eligibility assessment.

Please note that it is acceptable for a patient currently receiving first-line chemotherapy to register and consent for *K-RAS* testing if the treating consultant feels that PICCOLO may be a suitable option for the patient in the future. In this case, the *K-RAS* result will be held at the CTRU and if the patient subsequently decides to take part in PICCOLO there will be no delay to commencing trial treatment whist waiting for the *K-RAS* result.

When the patient has provided written consent for *K-RAS* testing, the research nurse will:

1. Call the registration line. A trial number will be allocated to the patient.
2. Ascertain the hospital(s) where previous colorectal cancer surgery was performed (e.g. the primary cancer resection), and obtain histology report(s) and sample number(s)
3. Contact the relevant histology department(s) to establish that they have tumour tissue blocks* and explain that they are needed urgently.
4. Fax the completed '*K-RAS* Sample Request Form' and a copy of the completed consent form to the histology department, with a copy of the same documents faxed to the CTRU.

**If the patient has previously participated in a clinical trial (e.g. COIN), tissue blocks may already be in a research lab. If this is the case, please contact the CTRU who will arrange direct transfer. If any other difficulties are encountered (eg histology department unable to release blocks) please contact the CTRU immediately.*

The '*K-RAS* Sample Request Form' includes instructions for the histology department sending the tissue blocks to the DNA laboratory. In most cases this will be:

PICCOLO Trial Laboratory
Gastrointestinal Cancer Translational Research,
Leeds Institute of Molecular Medicine,
Level 4, Wellcome-Brenner Building,
St James's University Hospital,
Leeds LS9 7TF, UK.

However, in some cases Units may have alternative arrangements for EGFR testing.

K-RAS mutation status will be notified directly from the PICCOLO Trial laboratory to the CTRU as soon as it is available.

Average turn-around from arrival of tissue blocks in the research laboratory is 4-5 working days: this and the time taken to retrieve the blocks should be taken into consideration when arranging the consent to participation in the trial/ randomisation visit (approx. 2 weeks in total).

***K-RAS* result**
Please note that the *K-RAS* result will be held in confidence by the CTRU and will not be made known to the site or patient.

5.2 Randomisation

A verbal explanation of the PICCOLO Patient Information Sheet and Consent Form document will be provided by the attending hospital staff for the patient to consider and a paper copy provided to the patient. This will include detailed information about the rationale, design and personal implications of the study. Following information provision, patients will have as long as

they need to consider participation (normally a minimum of 24 hours) and will be given the opportunity to discuss the trial with their family and healthcare professionals before they are asked whether they would be willing to take part in the trial. After the patient has provided written informed consent and has been confirmed as eligible the patient may be randomised.

Patients who fulfil the eligibility criteria, and have given signed informed consent, and whose *K-RAS* status has been determined as **mutation**, or whose *K-RAS* status is **unknown**, will be randomised on a 1:1 basis to receive either standard **Ir** or **IrCs**.

Patients who fulfil the eligibility criteria, have given signed informed consent, and whose *K-RAS* status has been determined as **wild-type** and **have not** previously received an anti-EGFR targeted therapy will be randomised on a 1:1 basis to receive either standard **Ir** or **IrPan**.

Patients who fulfil the eligibility criteria, have given signed informed consent, and whose *K-RAS* status has been determined as **wild-type** and **have** previously received an anti-EGFR targeted therapy will be randomised separately on a 1:1 basis to receive either standard **Ir** or **IrPan**.

Randomisations will be achieved using dynamic allocation incorporating a random element, via a computer-generated programme, to ensure treatment groups are well-balanced for the following patient characteristics, details of which will be required for randomisation:

- Randomising centre
- Previous treatment with oxaliplatin (no –*vs*– yes)
- Previous treatment with bevacizumab (no –*vs*– yes)
- Best response to any previous drug therapy (response/stable disease –*vs*– progressive disease alone –*vs*- unknown)
- Required dose reduction or delay for toxicity during previous therapy (yes –*vs*– no)
- WHO performance status (0 or 1 –*vs*– 2)

The person telephoning to randomise the patient should have the completed Randomisation Checklist in front of them at the time of telephoning as the following additional information will be required at randomisation:

- Patient's 5 digit trial no assigned at registration
- authorisation code and PIN
- basic patient details including initials, date of birth, hospital number, weight
- name of centre and centre code
- name of consultant and person making randomisation
- confirmation of eligibility (including date of written informed consent)

NB. The patient should be asked to complete the baseline quality of life questionnaire prior to randomisation.

5.2.1 Recruitment for Pharmacokinetics (PK) Sub-study

2-4 centres with facilities to perform pharmacokinetic sampling may register to participate in the PK Sub-study. At these centres only, patients who are randomised to receive IrCs may then be invited to consider participating in this sub-study, and provided with the supplementary PK Sub-study Patient Information Sheet and Consent Form document. The original PK sub-study Patient Information Sheet and Consent Form document should be filed in the supplementary PK sub-study Investigator Site File, a copy should be given to the patient and a further copy of the consent form section only sent to the Clinical Trials Research Unit (CTRU). A copy of the PK sub-study Patient Information Sheet and Consent Form document should be filed in the patient

hospital notes if required. It must be made clear that participation is entirely voluntary, and declining the PK Sub-study will in no way affect a patient's participation in the PICCOLO Trial.

Two consecutive treatment cycles of the first four (i.e. cycles 1 & 2, cycles 2 & 3, or cycles 3 & 4) will be nominated as PK Sub-study cycles. The centre will need to complete the PK Sub-study Registration Form and telephone the CTRU on the direct line for 24-hour randomisation in order to randomise the patient to receive, for these two cycles, IrCs and Ir in random order. All other treatment cycles will be with IrCs.

Details of the procedures for PK sampling, sample preparation, storage and transport are given in Appendix 4.

5.3 Baseline and reassessment CT scan

The baseline CT scan **must be within 4 weeks prior to the start of trial treatment**, so a repeat CT should be organised if required, remembering that randomisation cannot take place until *K-RAS* mutation status has been determined. Limited funding is available to contribute towards the cost of repeat baseline CT scans that are required only to meet the timelines set out in this protocol. Funding is available up to a limit of £150 per-patient at the time of writing this protocol. Please contact the CTRU for current funding availability.

Radiological reassessment is made **12 weeks after randomisation** ("12 week scan"), then at 12-weekly intervals, or sooner if clinically indicated, until disease progression ("follow-up scans"). It is recommended that the 12 week scan is pre-booked, at the time of starting treatment, **as near as possible to exactly 12 weeks after the date of randomisation** (with up to a further two weeks allowed in order to take account of practical issues).

If there is clinical evidence of disease progression at a time other than when radiological reassessment is due a CT scan should be carried out to confirm progression. A CT scan should always be carried out to confirm disease progression, unless there is a compelling reason not to.

Please note that copies of the baseline and the 12 week scan are required for external review. The preferred format is CD Rom, but hard-copy films are also acceptable. These should be forwarded to the PICCOLO team, CTRU, University of Leeds, 17 Springfield Mount, Leeds, LS2 9NG with their reports, as soon as possible after the 12 week scan has been performed. The scans and reports should be anonymised and labelled with the patient's trial number only. CTRU will provide pre-paid Special Delivery envelopes in which to send the CT scans for central review.

5.4 Non Randomisation

Participating clinicians/research nurses will be asked to complete a log of all patients screened for eligibility who are not randomised either because they are ineligible or because they decline participation. Anonymised information will be collected including:

- the reason not eligible for trial participation or
- eligible but declined
- age
- gender
- ethnicity

6. TREATMENT DETAILS

6.1 Routine pre-treatment tests

- On day 1 of each drug therapy cycle (= the day of irinotecan administration), or within five days prior, patients should undergo the following:
  - Medical history and physical examination, including assessment of WHO performance status
  - FBC, U&Es, magnesium, calcium and LFTs
    - If WBC < 3.0 x 10^9/l; neutrophils < 1.5 x 10^9/l, plts < 100 x 10^9/l; bilirubin > 1.5 ULN or estimated GFR < 50 ml/min, see section 6.4 for advice

- NB patients on **IrCs** may start their ciclosporin the day before these tests, but see note in section 6.4 if a treatment is delayed

NB if the patient is on a treatment break from irinotecan, panitumumab should be administered at 9mg/kg every three weeks (see section 6.2.5). When panitumumab is administered as a monotherapy the pre-treatment tests should be as detailed above.

6.2 Drug administration

Patients will receive treatment, once every three weeks, according to their randomisation allocation as follows:

- **Ir** (Control):
  - Irinotecan 350 mg/m^2 i.v. infusion (over 90* minutes)
    (Irinotecan 300 mg/m^2 if age >70 years or PS=2)

- **IrCs**:
  - Irinotecan 140 mg/m^2 i.v. infusion (over 40* minutes)
    (Irinotecan 120 mg/m^2 if age >70 years or PS=2)
    PLUS
  - Ciclosporin** 3mg/kg t.d.s. by mouth for 3 days starting in the morning the day before the dose of Irinotecan

- **IrPan**:
  - Panitumumab 9 mg/kg i.v. infusion (over 60* minutes), then
  - Irinotecan 350 mg/m^2 i.v. infusion (over 90* minutes),
    (Irinotecan 300 mg/m^2 if age >70 years or PS=2)

Please note that there is no requirement for a break between the Pan and Ir Infusions. If a patient's weight changes by > 10% from baseline the doses of all drugs should be re-calculated.

* Irinotecan and panitumumab infusion times may be reduced if well tolerated; see sections 6.2.2 and 6.2.4 respectively
** see section 6.2.3

Please note that any diluent used in this study must be an EU marketed product.

Dose capping
All dosing is to be determined solely by patient body surface area as calculated from actual body weight. Doses should not be capped.

6.2.1 Routine antiemetics, etc

In all three treatment arms:

- Immediately prior to irinotecan infusion:
  - dexamethasone 8mg i.v. + granisetron 3 mg i.v. or equivalents
- If cholinergic symptoms occur during/after irinotecan infusion:
  - atropine 0.3 mg s/c stat, and thereafter prophylactically for future cycles
- Following irinotecan infusion:
  - dexamethasone 4 mg orally, t.d.s. x 1 day; b.d. x 1 day and o.d. x 1 day
- Take-home medications to be prescribed routinely:
  - domperidone 10 mg t.d.s. p.r.n. for up to 7 days (please avoid metoclopramide as it may interact with Cs)
  - ensure patient has supplies of loperamide, and ciprofloxacin to use as required for diarrhoea, and instructions for their use (see section 6.4)
  - For patients on IrCs, the next cycle's prescription of ciclosporin should be issued

It is acceptable for centres to administer a variant of the above antiemetic schedule, as per local policy, but this should be applied to all three treatment arms.

6.2.2 Irinotecan infusion time

If treatment is tolerated without major acute cholinergic side-effects, the irinotecan infusion time may be reduced to 30 minutes (**Ir or IrPan**) or 15 minutes (**IrCs**) for subsequent cycles.

6.2.3 Ciclosporin administration

For patients receiving **IrCs**, the ciclosporin doses should be the same throughout the day and be spaced out as well as possible (e.g. 8am, 3pm and 10pm). See appendix 5 for details of ciclosporin dosing. The time of the irinotecan may fall close to the second ciclosporin dose on the second day, but the exact timing is not critical. Patients taking ciclosporin will be given a diary to record the time and number of doses taken.

Ciclosporin may be given as Neoral™ soft gelatin capsules or Neoral™ oral solution (Novartis) as preferred by the patient.

CAUTION: It is important to ensure that patients who have been taking ciclosporin at home are not accidentally given irinotecan at the full single-agent Ir dose. Each centre must ensure that appropriate checks are in place in medical, nursing and pharmacy systems to guard against this.
Prior to receiving irinotecan, patients should have taken 4-5 doses of ciclosporin (3 the previous day and 1 or 2 on the day of irinotecan, depending on the time of day the infusion is given). If the patient has taken at least 3 of the planned ciclosporin doses the irinotecan infusion may be given. The patient should be encouraged to take future ciclosporin doses as prescribed. If the patient has taken ≤2 doses of ciclosporin the irinotecan infusion should NOT be given. Ciclosporin should be re-prescribed and the irinotecan infusion re-scheduled for the earliest feasible opportunity (e.g. 2 days later).

6.2.4 Panitumumab administration

The dose of 9 mg/kg is calculated using actual body weight (the weight at baseline may be used throughout unless this changes by > 10% during the study) and is diluted in a minimum of 100 ml of pyrogen-free 0.9% sodium chloride solution. The final concentration should not exceed 10 mg/mL, therefore for patients weighing > 110 kg an infusion volume of > 100mL is required.
Panitumumab is administered IV by an infusion pump through a peripheral line or indwelling catheter **using a 0.22-micron in-line filter infusion set-up** over approximately 60 minutes. If well tolerated subsequent infusions can be reduced to 30 minutes but the infusion rate must not exceed 20 mg/min. In the unlikely event a subject's actual weight requires greater than 150ml volume infusion, panitumumab must be administered over approximately 90 minutes. Strict adherence to aseptic technique will be used during panitumumab preparation and administration. The bag should be labelled per site pharmacy/ward Standard Operating Procedures (SOPs) and promptly forwarded to the chemotherapy unit for infusion. Once diluted, panitumumab should be used within 6 hours if stored at room temperature or within 24 hours if refrigerated at 2-8°C.

There is no observation period for panitumumab.

6.2.5 Administration of panitumumab during chemotherapy breaks or delays

In the event that chemotherapy is to be delayed (e.g. for toxicity), panitumumab will be administered at a dose of 2.5 mg/kg on a weekly schedule until the beginning of the next chemotherapy cycle, when the standard schedule will be resumed.

For patients having a planned chemotherapy break (e.g. after cycle 4, in responding or stable patients) panitumumab will be administered as a single agent, at 9 mg/kg every 3 weeks, during the break.

6.3 Treatment Duration & Treatment Breaks

- The initial treatment period is 12 weeks followed by clinical and radiological re-assessment
- Patients with clinical benefit (radiological and symptomatic response or stabilisation) will be offered further treatment until disease progression or toxicity

- After the initial 12 weeks patients with stable or responding disease may have a planned treatment break of up to 6 weeks (i.e. omit up to 2 cycles of treatment, re-starting up to 9 weeks after the last irinotecan dose). In this event please note:
    a) there is no change in the scanning schedule i.e. patients should be scanned at 24, 36, 48 weeks etc post randomisation until progression
    b) patients on IrPan should continue to receive Pan as a single agent during the treatment break (see section 6.2.5)
    c) If clinical or radiological progression emerges during a break, the patient should resume their allocated treatment, provided that the treating consultant considers this to be in the patient's best interest. Patients on the IrPan arm who have received single-agent Pan during the break should re-start IrPan.

6.4 Management of Toxicity – Delays and Dose-reductions

General Rules:

- Except where specified below, non-haematological toxicity which persists at grade ≥2 when the next treatment is due requires a delay of one week.
- Except where specified below, if any non-haematological toxicity reaches grade ≥3, all subsequent treatment is given at a 20% reduced dose.
- For patients on IrPan who require a dose delay due to irinotecan toxicity, Pan is continued once-weekly (2.5mg/kg) until the Ir is resumed.
- For patients on IrCs who require a delay due to irinotecan toxicity, Cs is stopped, and a new full 3-day course started the day before Ir is resumed.

Management of specific toxicities:

- Acute cholinergic syndrome
    - Irinotecan may provoke acute cholinergic syndrome (diarrhoea, sweating, salivation, bradycardia, etc). This may start during the drug infusion or shortly after.
    - Treatment for cholinergic syndrome is atropine sulphate 0.3 mg s/c. This may be given according to local policy.

- Haematological toxicity
    - Check FBC before each cycle. Delay 1 week if WBC < 3.0 x 10^9/l, neutrophils < 1.5 x 10^9/l or platelets < 100 x 10^9/l. Only treat when FBC is above these limits.
    - If a delay of two consecutive cycles or 1 delay of ≥2 weeks occurs, reduce the irinotecan dose by 20% and continue at the lower dose for subsequent cycles unless further toxicity occurs
    - If a further delay(s) for myelotoxicity occurs despite a 20% dose reduction, a further dose reduction may be made, at the discretion of the treating clinician
    - In the event of febrile neutropenia reduce the irinotecan dose by 20% for all subsequent cycles

- Diarrhoea
    - Irinotecan delayed diarrhoea may, if untreated, become severe. Early intervention with high-dose loperamide is important.
    - Patients should be carefully instructed about this side effect, given a written information sheet, telephone contact numbers and supplies of loperamide and ciprofloxacin as per local practice. Care should be taken that out-of-hours staff answering patient queries are familiar with the protocol.

- Patients should start loperamide at the first loose stool: 4 mg, then 2 mg every 2 hours until 12 hours after the last loose stool (up to a maximum of 48 hours)
- If diarrhoea lasts > 24 hours, ciprofloxacin 500 mg bd should be added. If it lasts > 48 hours, or if the patient reports symptoms of dehydration, he/she should be admitted acutely for rehydration and further management (e.g. octreotide).
- After an episode of severe diarrhoea (e.g. requiring admission), delay until full recovery then resume at 20% reduced dose irinotecan for all subsequent cycles
- If diarrhoea from the previous cycle has not resolved by the time the next cycle is due, delay 1 week

- Hepatobiliary function
  - LFTs should be checked before each treatment cycle. Patients with serum ALP >5 x ULN or serum bilirubin in the range 1.5-3 x ULN require a 50% dose reduction of irinotecan. If the LFTs subsequently improve to below these limits, full-dose treatment may be resumed
  - Patients with serum bilirubin >3 x ULN should not receive irinotecan. If bilirubin subsequently falls, treatment may be resumed as above.

- Renal function
  - At the time of trial entry renal function must satisfy entry criteria. Subsequently re-check serum creatinine at each cycle. The patient should not receive treatment if estimated GFR falls below 30 ml/min.

- Hypomagnesemia Management
  - Plasma magnesium should be checked prior to each cycle. If below 0.7 mmol/l, start oral magnesium supplements (initially, we recommend oral Magnaspartate® [KoRa Healthcare], one 10mmol sachet t.d.s.). Serum magnesium should be maintained above 0.4 mmol/l at all times.

- Panitumumab dermatological toxicity
  - Over 90% of patients treated with panitumumab in previous trials developed skin or nail side-effects, usually a mild-to-moderate acneiform rash, similar to that seen during cetuximab therapy. This reached NCI CTC Grade 3 in 7% and resulted in discontinuation of the drug in 1% of patients.
  - All patients allocated to receive panitumumab should be forewarned that they are very likely to develop a rash. At the first development of a rash we recommend:
    - start an oral tetracyline, e.g lymecycline 408 mg b.d.
    - start topical emollients (e.g. E45®) and bath additives (e.g. Hydromol®).
  - Skin toxicities will be recorded as adverse events on the Treatment Case Report Form and will be graded using a modified version of NCI CTC version 3.0, as detailed in Appendix 2.

The following guidelines should be used for adjusting panitumumab in the event of skin or nail toxicity:

- If severe skin or nail toxicity occurs, the next panitumumab dose should be withheld and Ir administered alone. Examples of reasons for withholding a planned dose of panitumumab include:
  - Symptomatic skin- or nail-related toxicity requiring strong analgesia, systemic steroids, or felt to be intolerable by the patient

- Skin or nail infection requiring IV antibiotics or IV antifungals
- Need for surgical debridement
- Any skin- or nail-related serious adverse event

o After withholding a dose, panitumumab may be reintroduced at the next treatment cycle, at 75% of the original dose, provided:
- the adverse event has improved to ≤ Grade 2,
- the subject has recovered to the point where symptomatic skin or nail-related toxicity is no longer felt to be intolerable,
- systemic steroids or strong analgesics are no longer required,
- in the case of skin or nail infection, IV antibiotic or IV antifungal treatment is no longer required

o If the 75% dose reduction is tolerated, at the next cycle the panitumumab dose may be escalated to the original dose.

o If, after stopping and resuming panitumumab, a **second** withdrawal is required, any further reintroduction should be at 50% dose. If the 50% dose is tolerated, at the next cycle the panitumumab may be escalated to 75% of the original dose but should not be re-escalated to full dose.

o If, after stopping and resuming panitumumab, a **third** withdrawal is required the patient should be discontinued from receiving panitumumab

o If after withholding a dose of panitumumab, by the next treatment cycle:
- the adverse event has NOT improved to Grade 2
- the subject has NOT recovered to the point where symptomatic skin- or nail-related toxicity is no longer felt to be intolerable
- systemic steroids or strong analgesics are still required
- or, in the case of skin or nail infection, IV antibiotic or IV antifungal treatment is still required

…the patient should be discontinued from receiving panitumumab.

- Panitumumab non-dermatological toxicity

o In single-agent panitumumab studies, grade 1-2 diarrhoea, nausea, vomiting, abdominal pain and fatigue have been reported. It is therefore possible that panitumumab may contribute to these toxicities when given in combination with irinotecan.

o Toxicities will be recorded as adverse events on the Treatment Case Report Form (see section 8.2)

o Management of non-dermatological symptoms should be as advised above regardless of whether the patient is receiving or not receiving panitumumab. When indicated, dose reduction in irinotecan should be made without, in the first instance, changing the panitumumab dose.

- If non-dermatological toxicities persist despite irinotecan dose modification and, in the opinion of the investigator, panitumumab may be contributing, the following dose-reduction schedule should be used:
  - initially, withhold panitumumab for 1 treatment cycle
  - if toxicity recovers to ≤ grade 1, reintroduce panitumumab at 75% dose for the next cycle
  - as with dermatological toxicity, panitumumab may be escalated to 100% at the next cycle, If a second withdrawal is required any further re-introduction should be at 50% dose. If this is tolerated panitumumab may be escalated to, but should not exceed, 75% of the original dose.

- Panitumumab missed doses or delayed doses
  - In cases where panitumumab administration does not coincide with day 1 of chemotherapy (± 3 days), panitumumab should be administered at 2.5 mg/kg on a weekly schedule until the start of the next chemotherapy cycle, at which time the schedule will revert back to the 9-mg/kg every 3-week dose and schedule. Missed panitumumab dose will not be made up.

- Panitumumab infusion reactions
  - As with any administration of foreign protein, panitumumab carries a risk of hypersensitivity reactions. Since the start of panitumumab clinical studies to 19 June 2005 1575 patients have been exposed. From 20 June 2004 to 19 June 2005, 4 related hypersensitivity events (SUSARs) were reported.
  - Routine premedication is not recommended for panitumumab, although patients in PICCOLO receive i.v. dexamethasone as an antiemetic. In the event of a suspected infusion reaction, the infusion will be stopped. Depending on its severity the clinician may decide to withdraw panitumumab or continue, using a standard 24-hour premedication regimen.
  - In addition, any adverse events that are suspected to be infusion-related (even if they are not considered serious), must be reported, such as:
    - Cytokine release syndrome: fever, chills, rigors/shakes, and/or hypotension,
    - Hypersensitivity reaction: fever, chills, bradycardia/cardiac arrest, generalized urticaria, wheezing, bronchospasm, respiratory arrest, acute respiratory distress syndrome, and/or arthralgia/myalgia

- Ciclosporin toxicity
  - Some patients on IrCs experience mild clumsiness, tremor, nausea or abdominal griping during the three days of Cs treatment. Reassurance and general symptomatic treatments are normally sufficient; but if severely affected, reduce the Cs dose to 2 mg/kg t.d.s. for the remainder of the cycle and for all subsequent cycles.

- Some patients find the smell or taste of Cs foul. We recommend:
  - try switching from gelatin capsules to liquid or vice versa
  - "pop" the capsule blister-packs out-doors to avoid the smell
  - liquid may be mixed with orange juice to disguise the taste

- If a patient on IrCs is unable to tolerate Cs even with the above measures, they should cross over to standard Ir. However please see note in 6.5 regarding irinotecan dosing.

6.5 Cross-over

- Crossover involving ciclosporin:
  - Cross-over from **IrCs** to **Ir** should be avoided if possible, however if a patient cannot tolerate Cs this may be necessary. Please inform the CTRU.
    - the half-life of Cs is around 6 hours in most patients but may be as long as 20 hours. Therefore at least 1 week wash-out time must be allowed after the patient took their last Cs dose, before they receive standard-dose Ir.
  - Cross-over from **Ir** or **IrPan** to **IrCs** is not permitted
- Crossover involving Panitumumab:
  - Cross-over from **IrPan** to **Ir** should be avoided if possible, however if a patient cannot tolerate Pan this may be necessary. Please inform the CTRU.
  - Cross-over from **Ir** or **IrCs** to **IrPan** is not permitted.

6.6 Concomitant therapy

- Drugs which are potent inhibitors of CYP3A4 should be avoided, as they may affect irinotecan and/or ciclosporin metabolism. These include:
  - antifungals: ketoconazole; fluconazole; itraconazole
  - antibiotics: erythromycin; clarithromycin; norfloxacin
  - cardiac drugs: diltiazem; verapamil; amiodarone
  - antidepressants: fluvoxamine

- Many other drugs are substrates, inducers or mild inhibitors of CYP3A4. Interactions with irinotecan and ciclosporin are possible, but not known to be of major clinical significance. Use caution.

- Grapefruit juice contains potent CYP3A4 inhibitors and should be avoided for 3 days before and after each chemotherapy treatment

- Metoclopramide may increase Cs levels therefore we do not recommend metclopramide as an antiemetic for patients randomised to IrCs. Please use domperidone as an alternative.

6.7 Radiotherapy

Please avoid treating patients with radiotherapy during the initial 12 weeks of treatment; however if palliative radiotherapy is required for pain control this may be given. In this case radiotherapy should not be given during the 2 days before or the 5 after a dose of irinotecan. Full dose radical radiotherapy should not be used concurrently with PICCOLO treatment. Great care should be taken if radiotherapy is to the small or large bowel.

6.8 Pharmacokinetics Sub-study (see Appendix 4)

- In 20 consenting patients recruited to the IrCs arm in 2-4 centres with pharmacokinetics (PK) expertise, the plasma AUC of Ir and its metabolites will be compared during Ir and IrCs treatments during two consecutive cycles as follows:
    - For patients allocated **IrCs**, and participating in the PK Sub-study, two consecutive cycles during the first 4 cycles of therapy (i.e. cycles 1 & 2, cycles 2 & 3 or cycles 3 & 4) will be nominated as PK Sub-study cycles
    - For these two cycles the patient will receive, in random order, one cycle of IrCs and one cycle of standard Ir
    - PK blood sampling will be performed during the nominated cycles, as per Appendix 4

6.9 Drug Supply

Panitumumab will be supplied, packaged and labelled as per the requirements of the Medicines for Human Use (Clinical Trials) Regulations 2004.

Off-the-shelf supplies of irinotecan and ciclosporin will be used and labels will be provided for use at the time of dispensing.

6.10 Withdrawal of Treatment

In line with usual clinical care, cessation or alteration of regimens at any time will be at the discretion of attending clinicians or the patients themselves. All patients withdrawn from treatment or prescribed an alternative treatment will still attend for follow-up assessments unless unwilling to do so and Case Report Forms will continue to be completed.
If a patient explicitly states they do not wish to contribute further data to the study the CTRU should be informed in writing.

7. ASSESSMENTS/DATA COLLECTION

The trial consists of an initial 12-week treatment period, with further treatment until treatment failure (progression or toxicity). All patients will be followed up every 12 weeks until one year post-randomisation or death.

Trial data will be recorded by hospital research staff on the Case Report Forms (CRFs) and submitted to the CTRU at the University of Leeds. At the end of the trial, data will be securely archived at the CTRU and participating centres for a minimum of 15 years. Following

authorisation from the Sponsor, arrangements for confidential destruction will then be made. If a patient withdraws consent for their data to be used, it will be confidentially destroyed. It is the responsibility of each centre to retain copies of all completed CRFs and to maintain their file of essential trial documentation on site or at their designated archive facility.

7.1 Schedule of events

	Prior to registration	Prior to randomisation	Baseline (up to 4 weeks prior to treatment)	Day 1 of each cycle	12-Week Reassessment	Follow-up every subsequent 12 weeks, until one year post-randomisation
Informed consent for *K-RAS* test	X					
Informed consent to participate in the main trial		X				
Patient details (initials, date of birth, sex, hospital number)		X				
Serum or urine HCG[a]		X[b]				
Medical history[c]		X				
Physical examination[d]		X		X[e]		
Laboratory tests[f]		X		X[e]		
Concomitant diseases & medication		X		**Monitor throughout study**		
Quality of Life questionnaires		X[g]				
NCI-CTC[V3] grades				X	X	X
CT scan			X		X	X[h]
Adverse events				**Monitor throughout study**		
Survival/recurrence of disease				**Monitor throughout study**		

[a] If the patient is female and of child bearing potential
[b] Taken up to 72 hours prior to randomisation
[c] Including prior treatment with irinotecan, an anti-EGFR targeted therapy, oxaliplatin and bevacizamab
[d] Including height, weight, body surface area and WHO performance status
[e] Day 1 of each cycle or up to 5 days prior
[f] Including a combination of FBC, LFTs, U&Es, magnesium & calcium
[g] Further questionnaires at 12 and 24 weeks will be posted to the patients by the CTRU
[h] Or sooner if clinically indicated

7.2 Pre-randomisation Assessments

Post written informed consent and prior to randomisation, the following investigations and assessments will be carried out:

- Medical history including prior treatment with 5FU, capecitabine, oxaliplatin, bevacizamab or an anti-EGFR targeted therapy,, best response to previous drug therapy and dose delays/reductions for toxicity during previous drug therapy. Details of concomitant disease and medication will also be collected.
- Physical examination including height, weight, body surface area and WHO performance status
- Laboratory tests including FBC, LFTs, U&Es
- If the patient is female and of childbearing potential, a negative serum or urine HCG pregnancy test within 72 hours of trial entry
- Baseline Quality of Life questionnaires (for details please refer to section 7.8)

In addition the following data will be collected:

- Patient's GP name and address
- Patient's personal details and demographics

7.3 Baseline Assessments

The baseline CT scan must be carried out no more than 4 weeks prior to the start of trial treatment. Please note that trial treatment must start no more than 2 weeks post-randomisation. See section 5.3 for details.

7.4 Translational Research

N.B. This section applies to patients who have consented to participate in the associated translational research (optional points 11 and 12 on the PICCOLO Patient Information Sheet and Consent Form document).

A 10ml blood sample (in at EDTA tube) and a 20ml urine sample (in a plain collection tube) will be collected prior to the start of any trial treatment. The samples should be labelled with the patients' trial number only. Please send the blood and urine samples to the appropriate lab using the pre-paid, addressed safe boxes provided by the CTRU.

7.5 Treatment Assessments

Patients will be assessed clinically for symptoms and toxicity at the start of each drug therapy cycle i.e. 3-weekly. The CRF completed at these visits will include specific questions about the expected side-effects of the study drugs (using NCI-CTC version 3), plus any additional side effects, during the preceding cycle. Serious adverse events will be reported according to the Medicines for Human Use (Clinical Trials) Regulations 2004 (see section 8). On Day 1 of treatment (day of irinotecan infusion) the following investigations and assessments will be carried out:

- Height, weight, body surface area and WHO performance status (may be assessed up to 5 days prior to Day 1)
- Laboratory tests including FBC, LFTs, U&Es, magnesium and calcium (may be assessed up to 5 days prior to Day 1)
- Toxicity relating to the previous cycle assessed by NCI-CTC[V3] grades

In addition the following data will be collected:
- Cycle details including dose given, dose alteration/delay and reason

N.B. When a patient permanently stops trial treatment please fax a copy of the completed Treatment Stop Fax (section 7 of the Investigator Site File) to the CTRU.

7.6 12-Week Reassessment

At 12 weeks post-randomisation the following investigations and assessments will be carried out:
- Reassessment CT scan and response by RECIST criteria
- Toxicity assessed by NCI-CTC[V3] grades

In addition the following data will be collected:
- Clinical evidence of disease progression or not
- Treatment plan (i.e. continue or stop treatment)
- Details of any non-trial anti-cancer treatment received since randomisation
- Quality of Life questionnaires (for details please refer to section 7.9)

7.7 Progress and Follow-up Assessment

Follow-up data will be collected at 24 weeks post-randomisation and 12-weekly thereafter for all patients until 48 weeks post-randomisation, or until 6 weeks after the last PICCOLO treatment, whichever is later. The information required at these time-points includes late toxicity (adverse events occurring up to six weeks after the last dose of irinotecan), subsequent therapy and death.

The following investigations and assessments will be carried out:
- CT scan and response by RECIST criteria (if progression has not previously occurred).
- Toxicity assessed by NCI-CTC[V3] grades (up to 6 weeks after last study treatment dose)

In addition the following data will be collected:
- Patient status
- Summary of trial treatment since last assessment
- Details of any non-trial anti-cancer treatment received since last assessment
- Clinical evidence of disease progression or not
- Treatment plan
- Quality of Life questionnaires at 24 weeks post-randomisation (for details refer to section 7.9)

7.8 Serious Adverse Events (SAEs)

All SAEs must be recorded on the SAE CRF and be faxed immediately to the CTRU. See section 8 for further details.

7.9 Deaths

Death must be recorded on the Death Form. The date and cause of death will be collected. Death forms must be returned to CTRU within 7 days of the death being notified to the site team.

7.10 Quality of Life questionnaires

Quality of Life data will be collected in clinic prior to randomisation and via postal questionnaires at 12 and 24 weeks post-randomisation using the EORTC QLQ-C30, EQ-5D, Dermatology Life Quality Index (DLQI) and the MRC treatment value/acceptability supplement. To maintain confidentiality, patients will be provided in clinic with envelopes in which to seal their completed questionnaires. The CTRU will collect patients' full name and address on an individual Contact Details CRF in order to send out postal questionnaires. Prior to sending out postal questionnaires, the CTRU will confirm the patient's status and address with the hospital staff responsible for their care. Stamped addressed envelopes will be provided with the postal questionnaires. If a patient does not return the completed questionnaire within 2 weeks of posting one reminder questionnaire will be sent. If the patient does not respond to the reminder letter it will be deemed that the patient does not wish to complete the questionnaire. On receipt of a completed questionnaire the CTRU will post a letter of thanks to the patient.

7.11 Definition of End of Trial

The end of the trial is defined as the date of the last visit of the last patient undergoing the protocol based drug therapy. Follow-up will continue for one year after the last patient is randomised.

7.12 PK sub-study

In centres with appropriate facilities a subset of 20 patients will participate in the pharmacokinetic (PK) Sub-study (see Appendix 4). If a patient has consented to participate, ten 7ml blood samples will be collected prior to starting treatment, at 7 time points during the 6 hours following treatment and at 24 and 48 hours after treatment. The blood should be separated and the plasma stored at -40°C. The CTRU will arrange for the samples to be transported to the analysis laboratory. A contribution to PK sampling costs will be available (please contact CTRU for details).

8. PHARMACOVIGILANCE PROCEDURES

Within the trial, the following are defined as Investigational Medicinal Products (IMPs): irinotecan, ciclosporin and panitumumab.

8.1 General definitions

8.1.1 Adverse events

An adverse event (AE) is any untoward medical occurrence in a patient or clinical trial subject which does not necessarily have a causal relationship with this treatment and can include:

- any unintentional, unfavourable clinical sign or symptom
- any new illness or disease or the deterioration of existing disease or illness
- any clinically relevant deterioration in any laboratory assessments or clinical tests

8.1.2 Serious adverse events

A serious adverse event (SAE) is defined in general as any untoward medical occurrence or effect which:

- is fatal or life threatening*
- requires or prolongs hospitalisation
- is significantly or permanently disabling or incapacitating
- constitutes a congenital anomaly or a birth defect or
- may jeopardise the patient and may require medical or surgical intervention to prevent one of the outcomes listed above

* the term life threatening refers to an event in which the patient was at risk of death at the time of the event; it does not refer to an event which hypothetically might have caused death if it was more severe.

Where an SAE is deemed to have been related to an IMP used within this trial the event is termed a Serious Adverse Reaction (SAR).

8.1.3 Suspected unexpected serious adverse reactions

A suspected unexpected serious adverse reaction (SUSAR) is a serious adverse drug reaction which also demonstrates the following characteristic of being unexpected:
Unexpected – An adverse event, the nature OR severity of which is NOT consistent with the applicable product information (e.g. Investigator's Brochure for an unapproved investigational medicinal product, Summary of Product Characteristics for a marketed product).

8.2 Operational definition and reporting adverse events

From the first administration of trial treatment until 3 weeks after the last trial drug administration, all toxicities related to the underlying colorectal cancer or its treatment, whether observed by the investigator or reported by the patient, will be collected and recorded on a Case Report Form (CRF) following each cycle of treatment. The CTRU will provide details of all toxicities to the Data Monitoring and Ethics Committee (DMEC) for their review on a yearly basis.

Specifically, known toxicities associated with irniotecan, ciclosporin and panitumumab, as identified from the most recent Summary of Product Characteristics for inrinotecan and ciclosporin, and the most recent panitumumab Investigator Brochure will be collected. The following will therefore be included:

- Diarrhoea
- Nausea
- Vomiting
- Constipation
- Headache
- Lethargy
- Alopecia
- Dizziness
- Skin toxicity
- Abdominal pain
- Pyrexia
- Anaemia
- Neutropenia

- Thrombocytopenia
- Infection
- Nail toxicity
- Plus any other reported side-effects following each cycle of treatment of CTC grade ≥ 3

The following infusion related toxicities will also be collected:

- Fever
- Chills
- Respiratory
- Bradycardia/cardiac arrest
- Generalised urticaria
- Diarrhoea
- Arthralgia/myalgia
- Rigors/shakes
- Hypotension
- Plus any other reported infusion related toxicities following each cycle of treatment CTC grade ≥ 2

8.2.1 Pre-existing conditions

A pre-existing condition must not be reported as an AE unless the condition worsens significantly during the trial treatment.

8.2.2 Diagnostic and surgical procedures

Diagnostic and therapeutic non-invasive and invasive procedures, such as surgery, must not be reported as an AE. The medical condition for which the procedure was performed must be reported.

8.3 Operational definition serious adverse events (SAEs)

Death as a result of disease progression does NOT require reporting as an SAE, the Death Form should be completed and returned to the CTRU.

In addition, events NOT considered to be serious adverse events are hospitalisations for:

- routine treatment or monitoring of the studied indication, not associated with any deterioration in condition
- treatment, which was elective or pre-planned, for a pre-existing condition that is unrelated to the indication under study, and did not worsen
- admission to a hospital or other institution for general care, not associated with any deterioration in condition
- treatment on an emergency, outpatient basis for an event not fulfilling any of the definitions of serious given above and not resulting in hospital admission

8.4 Reporting serious adverse events

SAEs will be collected for all patients from the first trial treatment to 30 days after the last trial treatment. All SAEs must be recorded on the Serious Adverse Event Form and faxed to the CTRU on 0113 343 7985 within 24 hours of the research staff becoming aware of the event.

The local investigator must assign causality and expectedness to the SAE. A nominated individual at the CTRU will be responsible for the SAE procedures within the trial.

For each SAE, the following information will be collected:

- full details in medical terms with a diagnosis, if possible
- its duration (start and end dates; times, if applicable)
- action taken
- outcome
- causality, in the opinion of the investigator*
- whether the event would be considered expected or unexpected* (refer to the most recent and relevant Summary of Product Characteristics or Investigator Brochure)

*Assessment of causality and expectedness must be made by an authorised doctor. If an authorised doctor is unavailable, initial reports without causality and expectedness assessment should be submitted to the CTRU by a healthcare professional within 24 hours, but must be followed up by medical assessment as soon as possible thereafter. Please also indicate on the fax cover sheet when a medical assessment will be provided.

The local investigator and others responsible for patient care should institute any supplementary investigations of SAEs based on their clinical judgement of the likely causative factors. This may include seeking a further opinion from a specialist in the field of the SAE. If a patient dies, any post-mortem findings including histopathology must be provided to the CTRU. The CTRU will report all deaths to the DMEC for continuous safety review.

SAEs still present at the end of the study must be followed up at least until the final outcome is determined, even if it implies that the follow-up continues after the patient finishes the study treatment and when appropriate until the end of the planned period of follow-up.

The CTRU will inform the owner of panitumumab (Amgen Inc.) of all SAEs relating to patients on the IrPan arm within 1 working day of receipt, regardless of whether the event is suspected to be related to irinotecan or panitumumab.

The CTRU will report all SAEs to the main REC annually. All SAEs will also be reported to the DMEC for review approximately every three months to determine patterns and trends of events or identify safety issues.

The Investigator is responsible for reporting SAEs to their host institution, according to local regulations.

The Investigator does not need to inform the MHRA of SAEs related to the study drugs and expected, as this will be done annually by the CTRU. All adverse drug reactions suspected to be related to other licensed drugs used in standard care, for example anti-emetics, should be reported by the local investigator using the yellow card system. In agreeing to the provisions of this protocol, these responsibilities are accepted by the Investigator.

8.5 Reporting suspected unexpected serious adverse reactions (SUSARs)

All SAEs assigned by the local investigator as both *suspected* to be related to the trial drugs and *unexpected* are subject to expedited reporting. An event is unexpected when information is not consistent with the available product information or if they add significant information on the specificity or severity of an expected reaction (see Section 8.6).

The Chief Investigator (CI) or nominated individual will undertake urgent review of SUSARs within 24 hours of reporting and may request further information immediately from the patient's clinical team. The CI should not overrule the causality, expectedness or seriousness assessment given by the local investigator but may comment on these. The Investigator does not need to inform the MHRA of SUSARS. The CTRU will report all SUSARs to the MHRA and the main REC, within 7 days of the initial report being received, if the SUSAR resulted in death or was life-threatening, or within 15 days for any other unexpected serious adverse reaction. It is also recommended that the CTRU report all SUSARs related to irinotecan or ciclosporin to the marketing authorisation holders.

If information is incomplete at the time of initial reporting, the CTRU will request follow-up information, including information for analysis of causality, from the local investigator and will send the follow-up information to the MHRA and main REC within an additional 8 days for fatal or life-threatening SUSARs and as soon as possible for any others.

The CTRU will inform the owner of panitumumab (Amgen Inc.) of all SAEs relating to patients on the IrPan arm within 1 working day of receipt, regardless of whether the event is suspected to be related to irinotecan or panitumumab. Amgen will undertake urgent review of SUSARs and may request further information from the patient's clinical team via CTRU.

8.6 Expectedness of SAEs

The nature or severity of the event must be considered with reference to the applicable product information, for example the most recent Summary of Product Characteristics (SPC) or Investigator Brochure. If the nature or severity of the event is inconsistent with the SPC or IB the event must be classified as unexpected.

8.7 Expected SAEs for specific drugs/treatments

All investigators should refer to the Investigator Brochure for Panitumumab when determining whether a SAE is expected. The CTRU will ensure that any updates are circulated to all investigators. Any SAE's not listed in Appendix A of the Investigator Brochure should be classed as 'unexpected'.

The following table lists side effects/potential SAEs listed in the SPC for the irinotecan and ciclosporin and should therefore usually be considered as expected events and thus would not meet the criteria of SUSAR. The table should be used as a guide only and the most recent and relevant SPC must be referred to for more specific details and potential drug interactions.

Toxicity	Irinotecan	Ciclosporin
Biochemistry		
Alkaline phosphatase increase (mild to moderate)	✓	
Amylase increase (rare)	✓	
Bilirubin increase (mild to moderate)	✓	
Creatinine increase (mild to moderate)	✓	
Lipase increase (rare)	✓	
SGPT (grades 1-2)	✓	
SGOT (grades 1-2)	✓	
Transaminase increase (mild to moderate)	✓	

Toxicity	Irinotecan	Ciclosporin
Cardiovascular		
Cardio-circulatory failure (infrequent)	✓	
Hypertension	✓ (rare)	✓
Hypotension (infrequent)	✓	
Cutaneous		
Allergic rash		✓
Alopecia	✓	
Hypertrichosis		✓
Mild cutaneous reaction	✓	
Gastrointestinal		
Abdominal pain	✓	✓
Anorexia	✓	✓
Colitis (ischemic and ulcerative)	✓	
Delayed diarrhoea	✓	
Diarrhoea	✓	✓
Gastrointestinal haemorrhage	✓	
Gingival hyperplasia		✓
Intestinal obstruction	✓	
Intestinal perforation	✓	
Ileus	✓	
Mucositis	✓	
Nausea	✓	✓
Typhlitis	✓	
Vomiting	✓	✓
Haematological		
Anaemia	✓	✓
Febrile neutropenia	✓	
Haemolytic uraemic syndrome		✓
Hyperglycaemia		✓
Hyperkalaemia		✓
Hyperlipidaemia		✓
Hyperuricaemia		✓
Hypokalemia (rare)	✓	
Hypomagnesaemia		✓
Hyponatremia (rare)	✓	
Micro-angiopathic haemolytic anaemia		✓
Neutropenia	✓	
Sepsis	✓	
Thrombocytopenia	✓	✓
Neurotoxicity		
Encephalopathy/ demyelination (signs including convulsions, confusion, disorientationm decreased responsiveness, agitation, insomnia, visual disturbances, cortical blindness, coma, paresis, cerebellar ataxia)		✓
Headache		✓
Motor polyneuropathy		✓

Toxicity	Irinotecan	Ciclosporin
Neurotoxicity continued…		
Optic disc oedema (incl. papilloedema with possible visual impairment secondary to Benign Intracranial Hypertension) (very rare)		✓
Paresthesia	✓	✓
Tremor		✓
Dyspnoea	✓	
Interstitial pulmonary disease (uncommon)	✓	
General		
Acute cholinergic syndrome (early diarrhoea, abdominal pain, conjunctivitis, rhinitis, hypotension, vasodilatation, sweating, chills, malaise, dizziness, visual disturbances, myosis, lachrimation, increased salivation)	✓	
Allergic reaction	✓	
Anaphylactic/ anaphylactoid reaction	✓	
Asthenia	✓	
Dehydration	✓	
Fatigue		✓
Fever	✓	
Gynaecomastia		✓
Hepatic dysfunction		✓
Infection	✓	
Infusion reaction (fever, chills, rigors, shakes, hypotension)	✓	
Menstrual disturbances		✓
Muscular contraction/ cramps	✓	✓
Muscle weakness		✓
Myalgia		✓
Myopathy		✓
Oedema		✓
Pancreatitis		✓
Renal dysfunction		✓
Renal insufficiency (infrequent)	✓	
Transient speech disorders (very rare)	✓	
Weight increase		✓

8.7 Pharmacovigilance responsibilities

Local investigator:

1. Medical judgement in assigning to AEs:
   - Seriousness
   - Causality
   - Expectedness
2. To fax SAE form to CTRU within 24 hours of becoming aware and to provide further follow-up information as soon as available
3. To report SAEs to local committees in line with local arrangements

Chief Investigator (or nominated individual in CI's absence):

1. Chief Investigator to assign causality and expected nature of SAEs where it has not been possible to obtain local assessment
2. Chief Investigator will review all events assessed as SAEs in the opinion of the local investigator
3. Chief Investigator to review all events assessed as SUSARs in the opinion of the local investigator. In the event of disagreement between local assessment and Chief Investigator review with regards to SUSAR status, local assessment will not be overruled, but Chief Investigator may add comments prior to reporting to MHRA.

CTRU:

1. Report SAEs relating to patients on the IrPan arm to Amgen within 1 working day
2. Expedited reporting of SUSARs to MHRA and main REC within required timelines
3. Preparing annual safety reports to MHRA and main REC
4. Preparing safety reports for the DMEC at 3-monthly intervals for SAEs
5. Notifying Investigators of SUSARs which compromise patient safety
6. Distributing CIOMs line listings, as provided by Amgen quarterly, to Investigators and main REC on a regular basis

TSC:

1. Role in reviewing safety data and liaising with the DMEC regarding safety issues, as required

DMEC:

1. 3-monthly review of unblinded overall safety data to determine patterns and trends of events, or to identify safety issues, which would not be apparent on an individual case basis
2. Review of interim analysis

AMGEN INC:

1. Reporting all SUSARs related to panitumumab to the Global Regulatory Authorities, except to MHRA, within the required timelines
2. Providing the CTRU with quarterly Investigator CIOMs line listings
3. Review all SAEs relating to patients on the IrPan arm and inform CTRU of any upgrades to SUSAR

9. CRITERIA OF RESPONSE

Response to treatment will be assessed using the Response Evaluation Criteria in Solid Tumours (RECIST), please refer to Appendix 1. Responses will be confirmed by central review. External confirmation of RECIST response data will be performed on all baseline and 12-week scans.
The baseline and subsequent CT scans (film copies or CD data copies) will be forwarded to the CTRU for external radiological review (see section 5.3).

10. ENDPOINTS

10.1 Primary Endpoint

Ir vs IrCs comparison
- Proportion of patients progression-free 12 weeks after randomisation.

Ir vs IrPan comparison (patients with wild-type *K-RAS* status not previously receiving an anti-EGFR targeted therapy)
- Overall survival (OS) from randomisation

10.2 Secondary Endpoints

Ir vs IrCs comparison
- Proportion of patients free from treatment failure at 12 weeks
- Overall survival (OS) from randomisation
- Research nurse-assessed toxicity (NCI-CTC[V3] grades): maximum toxicity grade per patient; rate per cycle; all-cause mortality within 60 days of randomisation; toxicity of primary interest is grade 3+ diarrhoea within 12 weeks of randomisation;

Ir vs IrPan comparison (patients with wild-type *K-RAS* status not previously receiving an anti-EGFR targeted therapy)
- Proportion of patients progression-free 12 weeks from randomisation.
- Research nurse-assessed toxicity (NCI-CTC[V3] grades): maximum toxicity grade per patient; rate per cycle; all-cause mortality within 60 days of randomisation;

Ir vs IrCs and Ir vs IrPan (patients with wild-type *K-RAS* status not previously receiving an anti-EGFR targeted therapy) comparisons
- Progression-free survival (PFS) from randomisation
- Best response by RECIST criteria within 1-year follow-up from randomisation
- Patient-assessed symptom/QL/PA scores at 12 and 24 weeks

10.3 Exploratory Endpoints

Ir vs IrPan comparison (patients with wild-type *K-RAS* status previously receiving an anti-EGFR targeted therapy)

- Proportion of patients progression free 12 weeks after randomisation.
- Research nurse-assessed toxicity (NCI-CTC[V3] grades): maximum toxicity grade per patient; rate per cycle; all-cause mortality within 60 days of randomisation;

Ir vs IrPan comparison (patients randomised to receive Ir or IrPan under Protocol Version 1.0 who have mutant or unknown *K-RAS* status, regardless of previous anti-EGFR targeted therapy)

- Proportion of patients progression free 12 weeks after randomisation.
- Research nurse-assessed toxicity (NCI-CTC[V3] grades): maximum toxicity grade per patient; rate per cycle; all-cause mortality within 60 days of randomisation;

10.4 Choice of Primary Endpoint

Ir vs IrCs comparison

A non-inferiority trial requires a sensitive discriminator of efficacy. To use PFS for this purpose would require CT scanning at a frequency close to the difference being excluded, which in this trial is less than 6 weeks. Response rate is insensitive given the low rate of objective responses (<10%) with the standard therapy. OS is subject to influence by subsequent treatment. In contrast the proportion of patients free of progression at 12 weeks can be determined reliably with a single mandatory scan/assessment point, and the expected progression-free proportion of approximately 65% with standard therapy is a good basis for comparison.

Ir vs IrPan comparison (patients with willd-type *K-RAS* status not previously receiving an anti-EGFR targeted therapy)

The choice of OS for the primary endpoint for the +/- panitumumab comparison reflects the fact that for a novel therapy to be adopted into standard care, given known skin toxicity and likely cost, it would require a significant tangible benefit, and OS is objective and well accepted. Worthwhile treatment given at a relatively late stage in the disease should produce an OS effect. Furthermore this endpoint is reliable and avoids the need for very frequent scanning which is both arduous for patients and expensive. Hence our primary endpoint for the +/- panitumumab comparison is OS. The use of three secondary efficacy endpoints (PFS; RR; Progression-free at 12 weeks) ensures that a lesser efficacy benefit, requiring further phase III trials, would not go un-noticed.

11. STATISTICAL CONSIDERATIONS

11.1 Sample Size

Ir vs IrCs comparison

A non-inferiority design is proposed, based upon the proportion of patients progression-free at 12 weeks from randomisation. In a previous study in a similar patient population receiving irinotecan, median PFS was 4.2 months, with 63.3% of patients progression-free at 12 weeks.[38] There are no previous best supportive care (BSC) trials that have included regular CT scan assessments, however in a trial comparing irinotecan with BSC, at the time point that 63% of Ir

patients were alive, less than 40% of BSC patients were alive [40]. On this basis we have estimated that a similar proportion – 40% - of patients on BSC would be expected to be progression-free at 12 weeks applying standard WHO or RECIST criteria. On this basis irinotecan contributes at least 23% to the proportion of patients progression-free at 12 weeks. On the basis of clinician consensus IrCs will be defined as no worse than Ir if median PFS is at least 3 months with IrCs, equivalent to at least 52.7% of patients in the IrCs arm progression-free at 12 weeks. This corresponds to a reduction of no more than 10.6% in the proportion of patients progression-free at 12 weeks which is our non-inferiority margin; retaining more than half the effect that irinotecan has over BSC. The sample size calculation for the Ir vs. IrCs comparison remains the same since there is no data to suggest that *K-RAS* status is a predictive factor for Irinotecan or Ciclosporin.

We will conduct a one-sided test for non-inferiority.[39] With 80% power and a significance level of 2.5%, 680 patients are required to test for this degree of non-inferiority using a one-sided chi-squared test without continuity correction. To account for a 10% drop-out or 5% cross-over, 750 patients (375 per arm) would need to be randomised in total. This sample size also gives over 90% power for claiming non-inferiority of PFS using a one-sided modified log-rank test with significance level 2.5% (at least 273 PFS events are required to assess non-inferiority with 80% power), and allows detection of a clinically relevant relative reduction of 50% (from 20% with Ir to 10% with IrCs) in the proportion of patients experiencing grade 3+ diarrhoea within 12 weeks of randomisation, with over 90% power and at a 5% (two-sided) significance level, using a two-sided chi-squared test without continuity correction.

Although not primarily designed to detect superior efficacy for IrCs compared with Ir, this is a possible consequence of reduced toxicity if it leads to improved treatment duration. The proposed sample size will provide 80% power to detect an increase in median PFS of 1.1 months, or an increase in OS of 2.9 months with Cs, using a 2-sided log-rank test at the 5% significance level.

Ir vs IrPan comparison (patients with wild-type *K-RAS* status not previously receiving an anti-EGFR targeted therapy)

For the +/- panitumumab comparison, a superiority design is proposed, based upon overall survival (OS). Previous literature has found median OS for patients taking Ir to be between 9.2 and 10.8 months, mostly in patients pre-treated with only FU/LV.[38,40] Since many PICCOLO patients will have received FU/LV and oxaliplatin, we have assumed a slightly shorter median OS of 9 months in the control arm.

Since this comparison considers patients with wild-type *K-RAS* status only, for whom we expect the benefit of the addition of panitumumab to irinotecan to be increased, the study is now powered to detect a reduction in hazard rate of 30% with the addition of panitumumab, compared to no panitumumab. With 80% power and a significance level of 5%, 466 patients (246 deaths) are required to test for this degree of superiority using a 2-sided log-rank test, assuming patients are followed for a fixed length of time and that the hazard ratio is constant. 480 patients (240 per arm) will therefore be recruited to allow for loss to follow-up.

Previous anti-EGFR targeted therapy use

We expect that approximately 15% of patients recruited to PICCOLO will have previously been treated with an anti-EGFR targeted therapy. It is possible that there will be cross-resistance between anti-EGFR targeted therapies and other EGFR agents including panitumumab, and it is therefore important that the Ir vs. IrPan comparison compares only patients who have not

previously received anti-EGFR targeted therapies. This comparison will therefore include all patients who have not previously received an anti-EGFR targeted therapy who have been randomised to receive either Ir or IrPan (and who have wild-type *K-RAS* status).

Since previous anti-EGFR targeted therapy use is of no concern for the Ir vs. IrCs comparison, this analysis will include all patients randomised to receive either Ir or IrCs under Protocol Version 1.0, and all patients randomised to receive either Ir or IrCs via the Ir vs. IrCs randomisation in this amended protocol, regardless whether or not they have previously used anti-EGFR targeted therapy.

The two primary comparisons of Ir vs. IrCs and Ir vs. IrPan are essentially two independent comparisons[41]. We therefore aim to recruit 480 patients who have wild-type *K-RAS* status and who have not previously received an anti-EGFR targeted therapy (240 patients per arm) to the Ir vs. IrPan comparison. However, dependent upon the proportion of patients with unknown KRAS status, there may be some over-recruitment to this comparison. We expect approximately 15% of all patients recruited to this comparison to have previously received an anti-EGFR targeted therapy therefore the expected sample size for the exploratory analysis of Ir vs. IrPan for those patients previously receiving an anti-EGFR targeted therapy (and who have wild-type *K-RAS* status) is approximately 86 patients (43 patients per arm of Ir/IrPan).

***K-RAS* status**

We expect that the split between mutant *K-RAS* status and wild-type *K-RAS* status will be approximately 42:58, and that approximately 15% of patients will have unknown *K-RAS* status. The proportion of patients with unknown status will be monitored as this figure may vary. Only patients with wild-type *K-RAS* status will be randomised to the Ir vs. IrPan comparison. There will, however, be some patients randomized to IrPan via protocol Version 1.0 who will have either mutant or unknown *K-RAS* status. We anticipate that approximately 167 mutant or unknown *K-RAS* status patients will have been randomized to receive either Ir or IrPan under Protocol Version 1.0, of whom approximately 19 will have previously received an anti-EGFR targeted therapy. An exploratory analysis will be carried out on these patients.

Recruitment to both comparisons should continue until there are a total of at least 750 patients in the Ir versus IrCs comparison, and at least 480 wild-type *K-RAS* status, non-anti-EGFR pre-treated patients in the Ir versus IrPan comparison. The recruitment ratio to the Ir vs. IrCs comparison and the Ir vs. IrPan comparison will vary according to the proportion of patients with unknown *K-RAS* status, which we anticipate to be approximately 15%. Based on these assumptions, we expect the recruitment ratio to the Ir vs. IrCs comparison and the Ir vs. IrPan comparison to be approximately 50.7:49.3.

The total expected sample size, having already recruited 494 patients via Protocol Version 1.0, is therefore anticipated to be approximately **1324**, assuming 15% of patients have unknown *K-RAS* status at the time of randomisation, and assuming 15% of patients have previously received anti-EGFR targeted therapy.

The proportion of patients randomised with unknown *K-RAS* status, and the proportion of patients previously receiving anti-EGFR therapy, will be monitored in order to ensure that the assumptions made remain valid.

11.2 Planned Recruitment Rate

Target recruitment is 3 years. A total of 494 patients have been recruited under Protocol Version 1.0, therefore approximately 830 patients are still to be recruited in order to achieve the required sample size: an average of 46 per month (with 18 months recruitment remaining). Given that accrual to FOCUS was 60-65 patients per month, we feel this goal is realistic. Patients in the ongoing FOCUS2 trial (460 patients) may be eligible for PICCOLO after progression, as will patients in COIN (2400 patients). Recruitment will stop when 480 patients who have not previously received an anti-EGFR targeted therapy and who have wild-type *K-RAS* status have been recruited to the Ir vs. IrPan comparison, and when 750 patients have been recruited to the Ir vs. IrCs comparison.

12. STATISTICAL ANALYSIS

Statistical analysis is the responsibility of the CTRU Statistician. A final statistical analysis plan corresponding to Protocol Version 1.0 has been written in accordance with current CTRU standard operating procedures and has been finalised and agreed by the following people: the trial statistician and supervising statistician, the project manager and the Chief Investigator. Changes to this finalised analysis plan, and reasons for changes, will be documented. The above people will remain blinded to the data and will not have access to the complete CRFs.

All analyses will be conducted on the intention-to-treat (ITT) population (as defined in the statistical analysis plan), where patients will be included according to the treatment they were randomised to, and the per-protocol population (if necessary), where patients will be included according to the treatment they received. Patients defined as major protocol violators will be excluded from the per-protocol analysis. Major protocol violators were defined prior to the start of recruitment and any subsequent changes, and reasons for changes, will be documented. For the superiority endpoints the ITT analysis will be given primacy, however for the non-inferiority endpoints equal weighting will be given to both the ITT analysis and the per-protocol analysis, as the ITT is likely to be the least conservative approach when testing for non-inferiority. The safety population will consist of all patients who receive at least one dose of study treatment, and will be considered as necessary.

Overall survival is defined as the time from date of randomisation to date of death from any cause; patients with missing follow-up data, or who are still alive at the time of analysis, will be censored at the last date they were known to be alive. Progression-free survival is defined as the time between date of randomisation and date of disease progression or death from any cause. Patients with missing follow-up data, or who are alive and progression-free at the time of analysis, will be censored at the date they were last known to be alive and progression-free.

Analysis of 12-week assessments will include all patients assessed between 10 and 14 weeks from randomisation. A sensitivity analysis will be conducted to allow for patients assessed outside of this 2 week time frame. Analysis of response will be performed using the centrally reviewed outcome. If the centrally reviewed outcome is missing, the primary analysis will class the patient as being progression-free at 12 weeks (if alive), and a sensitivity analysis will be conducted using the outcome of the local assessment. If this is also missing, the patient will be classed as having progressed at 12 weeks in the sensitivity analysis.

A two-sided 5% significance level will be used for all superiority endpoints, and a one-sided 2.5% significance level will be used for all non-inferiority endpoints.

Safety data (adverse events and serious adverse events as detailed in Section 8) will be presented for all patients by treatment group and comparison, and by relationship to study drug, or underlying colorectal cancer.

Ir vs IrCs comparison

All patients randomised to receive either Ir or IrCs via protocol Version 1.0 or to the Ir vs. IrCs comparison under protocol Version 2.0 (i.e. mutant/unknown *K-RAS* status), regardless of previous anti-EGFR targeted therapy use, will be included in this comparison.

Primary Endpoint

Analysis will be based on the lower limit of the 95% confidence interval (CI) (one-sided type I error rate of 2.5%) of the difference in proportion of patients progression free at 12 weeks from randomisation between each treatment group. Logistic regression will also be used to adjust for the minimisation factors, previous anti-EGFR targeted therapy use and any other covariates identified to influence patients' prognosis. Treatment and covariate estimates with corresponding 95% CIs will be given. To account for any deaths from causes other than colorectal cancer which may occur within 12 weeks of randomisation, for whom no evidence of disease progression was found, sensitivity analyses will be conducted classifying these patients as progression-free (these patients will be classed as having progressive disease at 12 weeks in the main analysis). Sensitivity analysis will also be conducted to account for patients with missing data regarding progression at 12 weeks, using the local assessment or classifying these patients as with progressive disease, as appropriate. The primary analysis will be based on the lower limit of the confidence interval of the odds ratio for the proportion of patients progression free at 12 weeks, obtained using logistic regression which adjusts for the minimisation factors.

Secondary Endpoints

The difference in proportion of patients who are free from treatment failure, defined as clinical or radiological progression, death due to colorectal cancer or toxicity, or termination of treatment irrevocably, at 12 weeks from randomisation between treatment groups will be compared using a two-sided chi-squared test. Logistic regression will also be used to adjust for the minimisation factors, previous anti-EGFR targeted therapy use and any other covariates identified to influence patients' prognosis. Treatment and covariate estimates with corresponding 95% CIs will be given. Sensitivity analyses will be conducted to allow for any deaths from causes other than colorectal cancer within 12 weeks of randomisation. Sensitivity analysis will also be conducted to account for patients with missing data regarding treatment failure at 12 weeks, classifying these patients as with treatment failure.

Kaplan Meier curves for overall survival and progression-free survival will be calculated, and differences between the treatment groups compared using one-sided modified log-rank tests. Survival will also be compared using multivariate modelling, Cox's Proportional Hazards model if appropriate, to adjust for the minimisation factors, previous anti-EGFR targeted therapy use and other important prognostic factors. HRs and corresponding 95% CIs will be presented. Sensitivity analysis will be considered to account for missing data for PFS analysis.

Patients' best response rate[42] will be assessed by calculating the 95% CI of the difference in response rates between the two treatment groups. In addition, a one-sided chi-squared test for trend (or appropriate version) and logistic regression, to adjust for the minimisation factors,

previous anti-EGFR targeted therapy use and other prognostic factors, will be performed. Sensitivity analyses will be conducted to allow for any deaths from causes other than colorectal cancer, for whom no response status was observed.

Quality of life will be summarised using adjusted for baseline mean scores and 95% CIs for each EORTC QLQ-C30 domain, obtained from a multi-level repeated measures model, assuming missing data at random. Missing data patterns will be examined carefully, and if missing data patterns suggest data are missing not at random, analyses will also be carried out using pattern-mixture models, and data summarised using the Standardised Area Under the Curve. The MRC treatment value/acceptability supplement will be summarised descriptively with bar charts and summary tables for each question. The mean adjusted for baseline total Dermatology Life Quality Index score and scores for each domain will be presented with corresponding 95% confidence intervals.

To assess toxicity, the maximum grade per patient, rate per cycle and all cause mortality within 60 days of randomisation will be summarised descriptively for each treatment group. The difference between the two treatment groups in proportion of patients experiencing grade 3+ diarrhoea within 12 weeks of randomisation will be tested using a two-sided chi-squared test without continuity correction (or appropriate version), and 95% CI's will be presented. Logistic regression will also be used to adjust for the minimisation factors, previous anti-EGFR targeted therapy use and any other prognostic factors. Reasons for stopped treatment will be summarised.

Should non-inferiority of IrCs to Ir be shown, we will consider both treatment groups together, and conduct exploratory subgroup analyses comparing patients' outcome according to the clinical minimisation factors and previous anti-EGFR targeted therapy use.

Ir vs IrPan comparison (patients with wild-type *K-RAS* status not previously receiving an anti-EGFR targeted therapy)

Patients with wild-type *K-RAS* status randomised via the 'no previous anti-EGFR targeted therapy use' randomisation will be included in this comparison.

Primary Endpoint

Overall survival curves will be calculated using the Kaplan Meier method and compared using a 2-sided log-rank test. OS will also be compared between the control and experimental groups using multivariate modelling. Cox's proportional hazards model, if appropriate, will be used to adjust for the minimisation factors and other important prognostic factors. HRs and corresponding 95% CIs for OS will be presented. Primary analysis will be based on the multivariate analysis which adjusts for the minimisation factors.

Secondary Endpoints

The difference in proportion of patients who are progression-free at 12 weeks from randomisation between treatment groups will be compared using a two-sided chi-squared test. Logistic regression will also be used to adjust for the minimisation factors and any other covariates identified to influence patients' prognosis. Treatment and covariate estimates with corresponding 95% CIs will be given. To account for any deaths from causes other than colorectal cancer which may occur within 12 weeks of randomisation, for patients who have no evidence of disease progression, sensitivity analyses will be conducted classifying these patients as progression-free (these patients will be classed as with progressive disease at 12

weeks in the main analysis). Sensitivity analysis will also be conducted to account for patients with missing data regarding progression at 12 weeks, using local assessment data or classifying these patients as with progressive disease, as appropriate.

Kaplan Meier curves for progression-free survival will be calculated, and the difference between the treatment groups compared using a two-sided log-rank test. Survival will also be compared using multivariate modelling, Cox's Proportional Hazards model if appropriate, to adjust for the minimisation factors and other important prognostic factors. HRs and corresponding 95% CIs will be presented. Sensitivity analysis will be considered to account for missing data for PFS analysis.

Patients' best response rate[42] will be assessed by calculating the 95% CI of the difference in response rates between the two treatment groups. In addition, a two-sided chi-squared test for trend (or appropriate version) and logistic regression, to adjust for the minimisation factors and other prognostic factors will be performed. Sensitivity analyses will be conducted to allow for any deaths from causes other than colorectal cancer.

Quality of life will be summarised using adjusted for baseline mean scores and 95% CIs for each EORTC QLQ-C30 domain, obtained from a multi-level repeated measures model, assuming data to be missing at random. Missing data patterns will be examined carefully, and if missing data patterns suggest data are missing not at random, analyses will also be carried out using pattern-mixture models, and data summarised using the Standardised Area Under the Curve. The MRC treatment value/acceptability supplement will be summarised descriptively with bar charts and summary tables for each question. The mean adjusted for baseline total Dermatology Life Quality Index score and scores for each domain will be presented with corresponding 95% confidence intervals.

To assess toxicity, the maximum grade per patient, rate per cycle and all cause mortality within 60 days of randomisation will be summarised descriptively for each treatment group. Reasons for stopped treatment will be summarised.

It is possible that the presence of skin rash may be associated with the clinical effectiveness of anti-EGFr therapies[43]. For exploratory purposes it is therefore planned to examine the possibility that the treatment effect, as measured by best response rate, differs according to grade of acneiform rash. Best response rate will be assessed using ordered logistic regression adjusting for treatment, grade of skin toxicity and their interaction.

Ir vs. IrPan comparison (patients with wild-type *K-RAS* status previously receiving anti-EGFR targeted therapies)

Exploratory Analysis

The proportion of patients progression-free at 12 weeks from randomisation will be summarised for both the Ir and IrPan arms. 95% confidence intervals will also be reported.

To assess toxicity, the maximum grade per patient, rate per cycle and all cause mortality within 60 days of randomisation will be summarised descriptively for each treatment group. Reasons for stopped treatment will be summarised.

Ir vs. IrPan comparison (patients randomised to receive Ir or IrPan under Protocol Version 1.0 who have mutant or unknown *K-RAS* status, regardless of previous anti-EGFR targeted therapy)

Exploratory Analysis

The proportion of patients progression-free at 12 weeks from randomisation will be summarised for both the Ir and IrPan arms. 95% confidence intervals will also be reported.

To assess toxicity, the maximum grade per patient, rate per cycle and all cause mortality within 60 days of randomisation will be summarised descriptively for each treatment group. Reasons for stopped treatment will be summarised.

12.1 Interim Analysis

The DMEC met after approximately 100 patients had been randomised and completed 12-week follow-up in order to monitor the drop-out rate and the number of patients crossing over from IrCs to Ir, or from IrPan to Ir, in addition to monitoring safety data and recruitment. This gave an early indication of the tolerability of the novel regimens in this group of patients. In addition, the DMEC specifically reviewed the first cycle toxicity data for the first 100 patients randomised. The review allowed the DMEC to identify any increased toxicity rates for patients in the IrPan arm, compared to those in the Ir arm, which would provide supportive evidence for a protocol amendment to reduce the irinotecan dose for patients in the IrPan arm if necessary. The DMEC will meet at least annually thereafter. The DMEC will specifically consider the proportion of patients randomised with unknown *K-RAS* status, and the proportion of patients previously receiving anti-EGFR therapy, in order to ensure that the assumptions made for the sample size calculation are valid. Additionally, the DMEC will review the proportion of patients with wild-type *K-RAS* status who have been randomised to receive Ir and who go on to receive off-trial anti-EGFR therapy within three months of stopping PICCOLO therapy.

One formal statistical interim analysis is planned on the primary endpoint for the Ir vs IrCs comparison and the Ir ± panitumumab comparison, when the study is at least 18 months into recruitment and when at least half the required number of events in patients with wild-type *K-RAS* status who did not receive prior anti-EGFR targeted therapies (123 deaths) for the Ir vs. IrPan comparison have occurred between these two arms. This will be carried out by an independent statistician to the study. Specifically at the interim analysis, the DMEC, in light of the interim data, and of any advice or evidence they wish to request, will report to the Trial Steering Committee with a recommendation of trial adaptation or early closure of the corresponding arm of the study, without compromising the other comparison if the efficacy of Ir is significantly superior compared with IrCs ($p<0.05$) or IrPan ($p<0.001$) [44].

If the efficacy of IrCs is significantly superior ($p<0.005$) compared with Ir, or the efficacy of IrPan is significantly superior compared with Ir ($p<0.001$), then the DMEC will discuss with the TSC the need for trial closure or trial adaptation.

Since we are assessing inferiority and superiority of IrCs at the interim analysis, and non-inferiority of IrCs in the final analysis, we have not adjusted the α-levels of the CIs at the final analysis for this comparison. Different α-levels have been incorporated to reflect the relative importance of the interim analysis for superiority and inferiority claims.

Using the 0.1% significance level for the Ir vs. IrPan comparisons at the interim analysis controls the type I error using the methods of Haybittle-Peto[45]. By using such a conservative alpha at the interim analysis this allows us to maintain an overall critical value of 5% at the final analysis, for this comparison.[46]

There will be no formal statistical analysis of the exploratory endpoints for those patients who have previously received anti-EGFR targeted therapies, or have mutant or unknown *K-RAS* status, for the Ir vs. IrPan comparison.

If the DMEC recommends continuation of the trial they will assess cross-over rates specifically from IrCs, with a recommendation of whether it is necessary to increase the sample size to allow for higher cross-over. No other statistical analyses are planned until after the trial is closed to accrual. The first main report/publication of the trial will be prepared when, for the Ir/IrCs comparison every patient has reached 12 weeks follow-up, and for the ± panitumumab comparison when the required event rate of 246 deaths has occurred between the Ir and IrPan arms (patients with wild-type *K-RAS* status not previously receiving anti-EGFR targeted therapies) and when median follow up of at least one year has been reached. The database will be locked prior to the each main analysis. Longer-term endpoints for the Ir/IrCs comparison will be analysed when the required event rate of 273 PFS events across the Ir and IrCs arms, and median follow-up of one-year, has been reached.

13. DATA MONITORING

13.1 Data Monitoring and Ethics Committee

An independent DMEC has been established to review the safety and ethics of the trial. Detailed unblinded reports were prepared by the CTRU by an independent statistician for the DMEC after approximately 100 patients and 200 patients had been randomised and will be prepared approximately annually thereafter. The DMEC will work to a standard operating procedure prepared by the CTRU and based on the Damocles charter[47].

13.2 Data Monitoring

Data will be monitored for quality and completeness by the CTRU. Missing data will be chased until it is received, confirmed as not available, or the trial is at analysis. Reminders will be sent to patients if postal questionnaires are not returned on time. The CTRU will reserve the right to conduct source data verification which will be carried out by staff from the CTRU. Source data verification will involve direct access to patient notes at the participating hospital sites and the collection of copies of consent forms and other relevant investigation reports. A monitoring schedule will be established and agreed including primary endpoint and safety data. These will be seen by Amgen and will be considered and agreed by the DMEC, the Trial Steering Committee (TSC) and the Trial Management Group (TMG).

13.3 Clinical Governance Issues

To ensure responsibility and accountability for the overall quality of care received by patients during the study period, clinical governance issues pertaining to all aspects of routine management will be brought to the attention of the DMEC and, where applicable, to individual NHS Trusts.

14. QUALITY ASSURANCE & ETHICAL CONSIDERATIONS

14.1 Quality Assurance

The trial will be conducted in accordance with current EU Good Clinical Practice guidelines and through adherence to CTRU standard operating procedures.
The trial will be run under a Clinical Trial Authorisation granted from the MHRA.

14.2 Ethical Considerations

The trial will be performed in accordance with the recommendations guiding physicians in biomedical research involving human subjects adopted by the 18th World Medical Assembly, Helsinki, Finland, 1964, amended in 1996. Informed written consent will be obtained from the patients prior to randomisation into the study. The right of a patient to refuse participation without giving reasons must be respected. The patient must remain free to withdraw at any time from the study without giving reasons and without prejudicing his/her further treatment. The study will be submitted to and approved by a main Research Ethics Committee (REC) and the appropriate REC for each participating centre, prior to entering patients in the trial. The CTRU will provide the main REC with a copy of the final protocol, patient information sheet, consent forms and all other relevant study documentation.

15. PROTOCOL AMENDMENTS AND OTHER CHANGES IN STUDY CONDUCT

15.1 Protocol Amendments

Should any change be required to the final protocol, then a protocol amendment will be prepared and approved by the Internal Project Team and the Chief Investigator .

The Main Research Ethics Committee and the MHRA will be notified of all substantial amendments to the protocol and appropriate approval obtained prior to their implementation.

Deviations from the protocol and immediate implementation of a proposed protocol amendment should not be made except in medical emergency. In this instance the co-ordinating centre and the appropriate ethics committee should be advised immediately.

15.2 Changes in Study Conduct

A list of administrative changes will be maintained and this information forwarded to investigators and ethics committees (if applicable).

16. CONFIDENTIALITY

All information collected during the course of the trial will be kept strictly confidential. Information will be held securely on paper and electronically at the Clinical Trials Research Unit (CTRU). The CTRU will comply with all aspects of the 1998 Data Protection Act, operationally this will include:

- consent from patients to record personal details including name, date of birth, address and telephone number, NHS ID, hospital ID, GP name and address
- appropriate storage, restricted access and disposal arrangements for patient personal and clinical details
- consent from patients for access to their medical records by responsible individuals from the research staff or from regulatory authorities, where it is relevant to trial participation
- consent from patients for the data collected for the trial to be used to evaluate safety and develop new research
- patient name and address will be collected on a separate 'contact details' CRF and patient name will be collected on the consent form. All other data collection forms that are transferred to or from the CTRU will be coded with a trial number and will include two patient identifiers, usually the patient's initials and date of birth

An anonymised data set will be sent to Amgen who may use this data to support a license application within or outside the EU. The data protection laws outside the EU may be less stringent than within the EU; this is stated clearly in the patient information.

If a patient withdraws consent from further trial treatment but not from collection of data, their data and samples will remain on file and will be included in the final study analysis.

16.1 Archiving

At the end of the trial, data will be securely archived at the CTRU and participating centres for at least 15 years. Arrangements for confidential destruction or extension of the archiving period will then be made. If a patient withdraws consent for their data to be used, it will be confidentially destroyed immediately.

17. STATEMENT OF INDEMNITY

This trial is sponsored by the University of Leeds and the University of Leeds will be liable for negligent harm caused by the design of the trial. The NHS has a duty of care to patients treated, whether or not the patient is taking part in a clinical trial and the NHS (under standard NHS indemnity) remains liable for clinical negligence and other negligent harm to patients under this duty of care. As this is a clinician-led study there are no arrangements for no-fault compensation

18. STUDY ORGANISATIONAL STRUCTURE

18.1 Responsibilities

Chief Investigator – The Chief Investigator will have overall responsibility for the design and set-up of the trial, the investigational drug supply and pharmacovigilance within the trial.

Clinical Trials Research Unit (CTRU), University of Leeds - The CTRU will have responsibility for conduct of the trial in accordance with relevant GCP standards.

18.2 Operational Structure

Chief Investigator – The Chief Investigator is involved in the design, conduct, co-ordination and management of the trial

Trial Management Group – The TMG, comprising the Chief Investigator, Internal Project Team and Clinical Co-Investigators will be assigned responsibility for the clinical set-up, on-going management, promotion of the trial, and for the interpretation of results. Specifically the TMG will be responsible for (i) protocol completion, (ii) CRF development, (iii) obtaining approval from REC, (iv) submitting a CTA application and obtaining approval from the MHRA, (v) completing cost estimates and project initiation, (vi) appointing and facilitating the TSC and DMEC, (vii) reporting of serious adverse events, (viii) monitoring of screening, recruitment, treatment and follow-up procedures, (ix) auditing consent procedures, data collection, trial end-point validation and database development.

Clinical Trials Research Unit (CTRU), University of Leeds – The CTRU will provide set-up and monitoring of trial conduct to CTRU SOPs, ICH GCP and The Medicines for Human Use (Clinical Trials) Regulations 2004 (SI20041031) standards, randomisation design and service, database development and provision, protocol development, CRF design, trial design, source data verification, monitoring schedule and statistical analysis for the trial. In addition the CTRU will support REC and R&D submissions and clinical set-up, ongoing management including training, monitoring reports and promotion of the trial. The CTRU will be responsible for the day-to-day running of the trial including trial administration, database administrative functions, data management and safety reporting.

Trial Steering Committee (TSC) – The TSC will provide overall supervision of the trial, in particular trial progress, adherence to protocol, patient safety and consideration of new information. It will include an Independent Chair and not less than two other independent members. The Committee will meet annually.

Data Monitoring and Ethics Committee (DMEC) – The DMEC will review the safety and ethics of the trial by reviewing interim data during recruitment. The Committee will meet or communicate via teleconference at least annually.

19. PUBLICATION POLICY

The success of the study depends upon the collaboration of all participants. For this reason, credit for the main results will be given to all those who have collaborated in the trial, through

authorship and contributorship. Uniform requirements for authorship for manuscripts submitted to medical journals will guide authorship decisions. These state that authorship credit should be based only on substantial contribution to:

- conception and design, or acquisition of data, or analysis and interpretation of data
- drafting the article or revising it critically for important intellectual content
- and final approval of the version to be published

and that all these conditions must be met (www.icmje.org).

In light of this, the Chief Investigator and relevant senior CTRU staff will be named as authors in any publication. In addition, all collaborators will be listed as contributors for the main trial publication, giving details of roles in planning, conducting and reporting the trial.

To maintain the scientific integrity of the trial, data will not be released prior to the end of the trial, either for trial publication or oral presentation purposes, without the permission of the Trial Steering Committee or the Chief Investigator. In addition, individual collaborators must not publish data concerning their patients which is directly relevant to the questions posed in the trial until the main results of the trial have been published.

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Appendix 1. Response Evaluation Criteria in Solid Tumors (RECIST)

Eligibility

Only patients with measurable disease at baseline should be included in protocols where objective tumour response is the primary endpoint.

Measurable disease - the presence of at least one measurable lesion. If the measurable disease is restricted to a solitary lesion, its neoplastic nature should be confirmed by cytology/histology.

Measurable lesions - lesions that can be accurately measured in at least one dimension with longest diameter ≥20 mm using conventional techniques or ≥10 mm with spiral CT scan.

Non-measurable lesions - all other lesions, including small lesions (longest diameter <20 mm with conventional techniques or <10 mm with spiral CT scan), i.e., bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusion, inflammatory breast disease, lymphangitis cutis/pulmonis, cystic lesions, and also abdominal masses that are not confirmed and followed by imaging techniques; and.

- All measurements should be taken and recorded in metric notation, using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 4 weeks before the beginning of the treatment.
- The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up.
- Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). For the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

Methods of Measurement

- CT and MRI are the best currently available and reproducible methods to measure target lesions selected for response assessment. Conventional CT and MRI should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen and pelvis. Head and neck tumors and those of extremities usually require specific protocols.
- Lesions on chest X-ray are acceptable as measurable lesions when they are clearly defined and surrounded by aerated lung. However, CT is preferable.
- When the primary endpoint of the study is objective response evaluation, ultrasound (US) should not be used to measure tumor lesions. It is, however, a possible alternative to clinical measurements of superficial palpable lymph nodes, subcutaneous lesions and thyroid nodules. US might also be useful to confirm the complete disappearance of superficial lesions usually assessed by clinical examination.
- The utilization of endoscopy and laparoscopy for objective tumor evaluation has not yet been fully and widely validated. Their uses in this specific context require sophisticated equipment and a high level of expertise that may only be available in some centers. Therefore, the utilization of such techniques for objective tumor response should be restricted to validation purposes in

specialized centers. However, such techniques can be useful in confirming complete pathological response when biopsies are obtained.

- Tumor markers alone cannot be used to assess response. If markers are initially above the upper normal limit, they must normalize for a patient to be considered in complete clinical response when all lesions have disappeared.
- Cytology and histology can be used to differentiate between PR and CR in rare cases (e.g., after treatment to differentiate between residual benign lesions and residual malignant lesions in tumor types such as germ cell tumors).

Baseline documentation of "Target" and "Non-Target" lesions

- All measurable lesions up to a maximum of five lesions per organ and 10 lesions in total, representative of all involved organs should be identified as ***target lesions*** and recorded and measured at baseline.
- Target lesions should be selected on the basis of their size (lesions with the longest diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically).
- A sum of the longest diameter (LD) for *all target lesions* will be calculated and reported as the baseline sum LD. The baseline sum LD will be used as reference by which to characterize the objective tumor.
    - All other lesions (or sites of disease) should be identified as ***non-target lesions*** and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

Response Criteria

	Evaluation of target lesions
* Complete Response (CR):	Disappearance of all target lesions
* Partial Response (PR):	At least a 30% decrease in the sum of the LD of target lesions, taking as reference the baseline sum LD
* Progressive Disease (PD):	At least a 20% increase in the sum of the LD of target lesions, taking as reference the smallest sum LD recorded since the treatment started or the appearance of one or more new lesions
* Stable Disease (SD):	Neither sufficient shrinkage to qualify for PR nor sufficient increase to qualify for PD, taking as reference the smallest sum LD since the treatment started
	Evaluation of non-target lesions
* Complete Response (CR):	Disappearance of all non-target lesions and normalization of tumor marker level
* Incomplete Response/ Stable Disease (SD):	Persistence of one or more non-target lesion(s) or/and maintenance of tumor marker level above the normal limits

* Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions (1)

(1) Although a clear progression of "non target" lesions only is exceptional, in such circumstances, the opinion of the treating physician should prevail and the progression status should be confirmed later on by the review panel (or study chair).

Evaluation of best overall response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for PD the smallest measurements recorded since the treatment started). In general, the patient's best response assignment will depend on the achievement of both measurement and confirmation criteria

Target lesions	Non-Target lesions	Evaluation of non-target lesions	Overall response
CR	CR	No	CR
CR	Incomplete response/SD	No	PR
PR	Non-PD	No	PR
SD	Non-PD	No	SD
PD	Any	Yes or No	PD
Any	PD	Yes or No	PD
Any	Any	Yes	PD

- Patients with a global deterioration of health status requiring discontinuation of treatment without objective evidence of disease progression at that time should be classified as having "symptomatic deterioration". Every effort should be made to document the objective progression even after discontinuation of treatment.
- In some circumstances it may be difficult to distinguish residual disease from normal tissue. When the evaluation of complete response depends on this determination, it is recommended that the residual lesion be investigated (fine needle aspirate/biopsy) to confirm the complete response status.

Confirmation

- The main goal of confirmation of objective response is to avoid overestimating the response rate observed. In cases where confirmation of response is not feasible, it should be made clear when reporting the outcome of such studies that the responses are not confirmed.
- To be assigned a status of PR or CR, changes in tumor measurements must be confirmed by repeat assessments that should be performed no less than 4 weeks after the criteria for response are first met. Longer intervals as determined by the study protocol may also be appropriate.
- In the case of SD, follow-up measurements must have met the SD criteria at least once after study entry at a minimum interval (in general, not less than 6-8 weeks) that is defined in the study protocol

Duration of overall response

- The duration of overall response is measured from the time measurement criteria are met for CR or PR (whichever status is recorded first) until the first date that recurrence or PD is objectively documented, taking as reference for PD the smallest measurements recorded since the treatment started.

Duration of stable disease

- SD is measured from the start of the treatment until the criteria for disease progression are met, taking as reference the smallest measurements recorded since the treatment started.
- The clinical relevance of the duration of SD varies for different tumor types and grades. Therefore, it is highly recommended that the protocol specify the minimal time interval required between two measurements for determination of SD. This time interval should take into account the expected clinical benefit that such a status may bring to the population under study.

Response review

- For trials where the response rate is the primary endpoint it is strongly recommended that all responses be reviewed by an expert(s) independent of the study at the study's completion. Simultaneous review of the patients' files and radiological images is the best approach.

Reporting of results

- All patients included in the study must be assessed for response to treatment, even if there are major protocol treatment deviations or if they are ineligible. Each patient will be assigned one of the following categories: 1) complete response, 2) partial response, 3) stable disease, 4) progressive disease, 5) early death from malignant disease, 6) early death from toxicity, 7) early death because of other cause, or 9) unknown (not assessable, insufficient data).

- All of the patients who met the eligibility criteria should be included in the main analysis of the response rate. Patients in response categories 4-9 should be considered as failing to respond to treatment (disease progression). Thus, an incorrect treatment schedule or drug administration does not result in exclusion from the analysis of the response rate. Precise definitions for categories 4-9 will be protocol specific.

- All conclusions should be based on all eligible patients.

- Subanalyses may then be performed on the basis of a subset of patients, excluding those for whom major protocol deviations have been identified (e.g., early death due to other reasons, early discontinuation of treatment, major protocol violations, etc.). However, these subanalyses may not serve as the basis for drawing conclusions concerning treatment efficacy, and the reasons for excluding patients from the analysis should be clearly reported.

- The 95% confidence intervals should be provided.

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Appendix 2. Toxicity criteria

NCI CTC Toxicity criteria (v3.0). For full list see http://ctep.cancer.gov/reporting/ctc.html

Toxicity	0	1	2	3	4
NAUSEA	None	Loss of appetitie without alteration in eating habits	Oral intake decreased without significant weight loss, dehydration or malnutrition; IV fluids indicated < 24hrs	Inadequate oral caloric or fluid intake; IV fluids, tube feedings, or TPN indicated ≥ 24hrs	Life-threatening consequences
VOMITING	None	1 episode in 24 hours	2-5 episodes in 24 hours; IV fluids indicated < 24hrs	≥ 6 episodes in 24 hours; IV fluids, or TPN indicated ≥ 24hrs	Life-threatening consequences
ANOREXIA	None	Loss of appetite without alteration in eating habits	Oral intake altered without significant weight loss or malnutrition; oral nutritional supplements indicated	Associated with significant weight loss or malnutrition; IV fluids, tube feedings or TPN indicated	Life-threatening consequences
ALOPECIA	Normal	Thinning or patchy	Complete	-	-
PAIN	None	Mild pain not interfering with function	Moderate pain: pain or analgesics interfering with function, but not interfering with ADL	Severe pain: pain or analgesics severely interfering with ADL	Disabling
STOMATITIS	None	Minimal discomfort, intervention not indicated	Symptomatic, medical intervention indicated but not interfering with ADL	Stool incontinence or other symptoms interfering with ADL	Symptoms associated with life-threatening consequences
DIARRHOEA (patients without colostomy)	None	Increase of <4 stools/day over baseline	Increase of 4-6 stools/day over baseline; IV fluids indicated < 24hrs	Increase of ≥ 7 stools/day; incontinence; IV fluids ≥ 24hrs; hospitilisation	Life-threatening consequences (e.g., haemodynamic collapse)
DIARRHOEA (patients with a colostomy)	None	Mild increase in ostimy output compared with baseline	Moderate increase in ostomy output compared with baseline, not interfering with ADL	Severe increase in stoma output compared to baseline, interfering with ADL	Life-threatening consequences (e.g., haemodynamic collapse)
LETHARGY	None	Mild fatigue over baseline	Moderate or causing difficulty performing some activities	Severe fatigue interfering with ADL	Disabling
HB	Within normal limits	10.0g/dl - normal	8.0 - 9.9g/dl	6.5 - 7.9g/dl	<6.5g/dl
PLATELETS	Within normal limits	$75x10^9/1$ - normal	50 - $74x10^9/1$	25 – $49x10^9/1$	<$25x10^9/1$
WBC	Within normal limits	$3.0x10^9/1$- normal	2.0 - $2.9x10^9/1$	1.0 - $1.9x10^9/1$	<$1.0x10^9/1$
NEUTROPHILS	Within normal limits	$1.5x10^9/l$ – normal	1.0 - $1.4x10^9/1$	0.5 - $0.9x10^9/1$	<$0.5x10^9/1$

The skin toxicity criteria below are modified from the NCI CTC Toxicity criteria (v3.0).

Toxicity	0	1	2	3	4
NAIL CHANGES	None	Discolouration, ridging. Paronychia: intervention not indicated	Partial/complete nail loss; nailbed pain. Paronychia: intervention indicated	Interfering with activities of daily living (ADL)	──
SKIN ERYTHEMA	None	Painless erythema	Painful erythema	Erythema with desquamation*	Life-threatening; disabling
PRURITIS (ITCHING)	None	Mild or localized	Intense or widespread	Intense or widespread and interfering with ADL	──
ACNEIFORM RASH	None	Intervention not indicated	Intervention indicated	Causing pain requiring narcotic analgesics, ulceration, or desquamation*	──
NON-ACNE-IFORM RASH	None	Macular or papular eruption or erythema without associated symptoms	Macular or papular eruption or erythema with pruritis or other symptoms; localized desquamation* or other lesions covering < 50% of body surface area (BSA)	Severe, generalized erythroderma or macular, papular or vesicular eruption; desquamation* Covering ≥ 50% BSA	Generalized exfoliative, ulcerative, or bullous dermatitis
SKIN ULCERATION	None	──	Superficial ulceration < 2 cm size; local wound care; medical intervention indicated	Ulceration ≥ 2 cm size; operative debridement, primary closure or other invasive intervention indicated (eg HBO_2)	Life-threatening; major intervention indicated (eg complete resection, tissue reconstruction, flap, or grafting)

**Desquamation is defined as sloughing of skin and does not apply to dry flaking skin.*

Appendix 3. Guide for handling panitumumab

Panitumumab (ABX-EGF) Packaging and Formulation

Panitumumab (ABX-EGF) will be packaged by Amgen and distributed using Amgen's clinical trial drug distribution procedures. Each vial of panitumumab will contain 10 mL of a sterile protein solution containing a 20-mg/mL solution of panitumumab. The vials will contain approximately 200 mg of study drug and are for single dose use only. Boxes of panitumumab will contain 12 vials of study drug. Amgen will conduct initial shipments of study drug and maintain on-going re-supply.

Panitumumab Labeling

Each vial of study drug will be labeled with the following: Panitumumab (ABX-EGF) 20 mg/mL, 10.0 mL I.V., Immunex, 2°C - 8°C. In addition the labels will display the following cautionary statement: 'for clinical trial use only, keep out of reach and sight of children, keep to the advised dosage'.

Panitumumab Storage

The supplied investigational drug must be stored at 2-8°C in a secured area upon receipt. As study drug contains no preservatives, vials are designed for single use only. Exposure of the material to excessive temperature above or below this range should be avoided. Do not allow study drug to freeze and do not use if contents freeze in transit or in storage. If vials fall out of specified temperature requirement please contact the sponsor or its designee for instructions.

Records of the actual storage conditions during the period of the study must be maintained (ie, records of the date and time and initials of person checking, and the "working day" temperatures of the refrigerator used for storage of trial supplies, continuous temperature recordings, or regularly maintained temperature alarm systems used in conjunction with temperature recording.

Panitumumab Preparation

NOTE: Panitumumab is a protein and should be handled gently to avoid foaming, which may lead to denaturation of the protein product.

The pharmacist, or designated ward nurse, using aseptic techniques will prepare panitumaumab for infusion. The dose of panitumumab is based upon the subject's baseline weight, and need not be recalculated unless the weight changes more than 10% from the baseline. The calculated amount of panitumumab will be removed from the vials and added to approximately 100 mL of pyrogen-free 0.9% sodium chloride solution, USP/PhEur. The total concentration infused should not exceed 10 mg/mL of panitumumab. For patients weighing over 111 kg the volume of saline will need to be increased as needed to ensure the maximum concentration of the diluted solution does not exceed 10 mg/mL. Once diluted the product should be used within 6 hours of preparation if stored at room temperature, or within 24 hours of dilution if stored refrigerated at 2-8°C. The study drug will be will be labeled per site pharmacy SOPs. The bag should be labeled per site pharmacy SOPs and promptly forwarded to the chemotherapy unit for infusion.

Supply and Return of Panitumumab

At study initiation and as needed thereafter, study drug will be shipped to a responsible person (ie, a pharmacist) at the Investigator's institution, who will check the amount and condition of the drug and enter these data into the Proof of Receipt Form. The Proof of Receipt Form should then be faxed to CTRU and Amgen. The original Proof of Receipt Form will be retained at the site. At the end of the

study, or as directed, all study drug supplies, including unused, partially used, or empty containers, will be retained at the site until a representative of the CTRU can audit drug accountability records and arrange for the destruction or return of used and unused study medication to Amgen (or alternative disposition if authorized by Amgen and in compliance with applicable regulatory requirements).

Panitumumab Accountability

An investigational product accountability record for the investigational product panitumumab must be kept current and should contain:

- dates and quantities of investigational product received from Amgen
- manufacturing batch or lot numbers for product received
- subject's identification (subject number and initials)
- date and quantity of investigational product dispensed (and remaining, if from individual subject drug units)
- initials of the dispenser
- dose preparation records
- date and quantity of drug returned to the Investigator/pharmacy, if appropriate

Any discrepancies must be documented and subsequently reported to the sponsor immediately. Where applicable, describe whether the returned investigational product will need to be counted.

At the end of the study, a final investigational product reconciliation statement must be completed and provided to the sponsor or its designee.

All inventories of study drug must be made available for inspection by the CTRU representative(s) and regulatory agency inspector(s). The Investigator is responsible for the accountability. Further details of this and other pharmacy procedures will be provided in the investigator and pharmacy files.

Appendix 4. Pharmacokinetics (PK) Sub-study

The regimen of IrCs used in this trial has been chosen, based on PK data from previous studies, to generate the same systemic exposure to irinotecan and its active metabolite SN38 (measured as AUC – the area under their plasma concentration/time curves) as standard single-agent irinotecan. In order to confirm whether this is in fact achieved, 20 patients in the IrCs arm are being invited to participate in the PK Sub-study.

Any centre with facilities and experience in PK may register for the PK Sub-study. In those centres, all patients randomised to the IrCs arm will then be invited to participate in the PK Sub-study; patients are however free to decline participation without affecting any other aspect of their trial participation or medical care. Recruitment to the PK Sub-study will be discontinued when the required target of 20 patients have completed two PK study days.

PK Sub-study entry procedure

- Patients will be asked to sign the PK Sub-study Consent Form
- Two consecutive treatment cycles during the first four will be nominated as 'PK Sub-study cycles'. These will normally be cycles 1 & 2, however the PK Sub-study may be deferred to cycles 2 & 3, or cycles 3 & 4, if these are more convenient for the patient and Centre.
- The centre will complete the PK Sub-study Registration Form and telephone the CTRU on 0113 343 7957 to obtain the randomised allocation i.e. **allocation to receive, in random order, one of these two cycles as IrCs and one as standard single-agent irinotecan.**
- Patients planned to receive Ir at the lower dose because of age ≥70 or PS=2 (see section 6.2) may still take part in the PK Sub-study. In this case the lower dose will be used for both the IrCs study cycle (120 mg/m^2) and the standard Ir study cycle (300 mg/m^2).
- Any patient requiring irinotecan dose reduction for toxicity before completion of the two PK study cycles will be removed from the PK Sub-study.

PK Sub-study Sampling Procedure

- The patient will need to have blood samples taken 6, 24 and 48 hours after the end of irinotecan infusion. For this reason, on PK study days we recommend that the patient is booked to attend in time for administration of irinotecan early (e.g. 10 am) to avoid late evening sampling.
- For the IrCs cycle we recommend you telephone the patient 2 days before to remind them to start their Cs at 8 am the day before attending for irinotecan.
- For the standard Ir cycle, double-check that the patient has **NOT** taken Cs (see 6.2.3).
- Before starting the irinotecan infusion, insert a second venous cannula for blood sampling in the arm opposite to that to be used for irinotecan administration. For each PK sample, 2 ml blood is discarded from the cannula, then 7ml blood is withdrawn into a Lithium-Heparin container and placed on ice. The cannula is flushed with normal saline.
- Transport the sample on ice to the refrigerated centrifuge immediately. Centrifuge within 10 minutes of of withdrawal, by centrifugation at 1000g for 10mins at 4^{0}C. Transfer plasma into the labelled aliquot tubes provided and store at –40^{o}C or lower pending transportation to the analytical laboratory.

PK Sample times

- Planned sample times are:
    - baseline (immediately before starting Ir infusion)
    - end of irinotecan infusion (= 90 minutes for Ir and 40 minutes for IrCs*). All subsequent samples are timed from the end of irinotecan infusion, as follows:
    - 15 minutes after end of irinotecan infusion
    - 30 minutes after end of irinotecan infusion
    - 60 minutes after end of irinotecan infusion
    - 2 hours after end of irinotecan infusion
    - 4 hours after end of irinotecan infusion
    - 6 hours after end of irinotecan infusion
    - 24 hours after end of irinotecan infusion
    - 48 hours after end of irinotecan infusion

*Please note that patients on the PK Sub-study should receive their irinotecan infusions at full length for the two designated sub-study cycles. Patients may receive irinotecan at a reduced infusion length for subsequent cycles.

- Whilst these are the planned sample times, it is the *actual* sample times which will be used for PK modelling. Therefore, please record the exact time of each sample, to the nearest minute, on the PK Sub-study CRF.
- Also record on the CRF if any problems are encountered with a sample, eg a delay in centrifugation or freezing. This will allow “outlying” data-points to be interpreted.

After completion of the PK Sub-study

Please notify the CTRU that the PK Sub-study is complete. They will arrange for the samples to be transported to the analysis laboratory.

Appendix 5. Ciclosporin dosing

Dose 3mg/kg x 3 a day

Weight of patient (kg)	Calculated dose @ 3 mg/kg (mg)	Ciclosporin capsules				Actual dose (mg)
		10mg	25mg	50mg	100mg	
40	120	2			1	120
41	123		1		1	125
42	126		1		1	125
43	129	3			1	130
44	132	3			1	130
45	135	1	1		1	135
46	138	4			1	140
47	141	4			1	140
48	144	2	1		1	145
49	147			1	1	150
50	150			1	1	150
51	153			1	1	150
52	156	1		1	1	160
53	159	1		1	1	160
54	162	1		1	1	160
55	165	4	1		1	165
56	168	2		1	1	170
57	171	2		1	1	170
58	174		3		1	175
59	177		3		1	175
60	180	3		1	1	180
61	183	1	1	1	1	185
62	186	1	1	1	1	185
63	189	4		1	1	190
64	192	4		1	1	190
65	195	4		1	1	190
66	198				2	200
67	201				2	200
68	204				2	200
69	207	1			2	210
70	210	1			2	210
71	213	1			2	210
72	216	2			2	220
73	219	2			2	220
74	222	2			2	220
75	225		1		2	225
76	228	3			2	230
77	231	3			2	230
78	234	1	1		2	235
79	237	4			2	240
80	240	4			2	240
81	243	2	1		2	245
82	246			1	2	250

Weight of patient (kg)	Calculated dose @ 3 mg/kg (mg)	Ciclosporin capsules				Actual dose (mg/kg)
		10mg	25mg	50mg	100mg	
83	249			1	2	250
84	252			1	2	250
85	255			1	2	250
86	258	1		1	2	260
87	261	1		1	2	260
88	264	1		1	2	260
89	267	2		1	2	270
90	270		3		2	270
91	273		3		2	275
92	276		3		2	275
93	279	3		1	2	280
94	282	3		1	2	280
95	285	3		1	2	280
96	288	4		1	2	290
97	291	4		1	2	290
98	294				3	300
99	297				3	300
100	300				3	300
101	303				3	300
102	306	1			3	310
103	309	1			3	310
104	312	1			3	310
105	315	1			3	310
106	318	2			3	320
107	321	2			3	320
108	324		1		3	325
109	327		1		3	325
110	330	3			3	330
111	333	3			3	330
112	336	4			3	340
113	339	4			3	340
114	342	4			3	340
115	345	4			3	340
116	348			1	3	350
117	351			1	3	350
118	354			1	3	350
119	357	1		1	3	360
120	360	1		1	3	360

Where the patient is taking liquid ciclosporin round the dose to the nearest 10mg.