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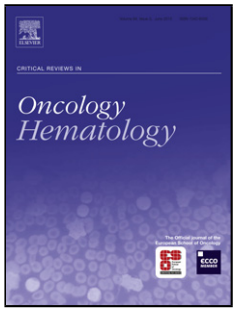

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A minimum core outcome dataset for the reporting of preclinical chemotherapeutic drug studies: Lessons learned from multiple discordant methodologies in the setting of colorectal cancer

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Abstract

In vivo studies in animal models are critical tools necessary to study the fundamental complexity of carcinogenesis. A constant strive to improve animal models in cancer exists, especially those investigating the use of chemotherapeutic effectiveness. In the present systematic review, colorectal cancer (CRC) is used as an example to highlight and critically evaluate the range of reporting strategies used when investigating chemotherapeutic agents in the preclinical setting. A systematic review examining the methodology and reporting of preclinical chemotherapeutic drug studies using CRC murine models was conducted. A total of 45 studies were included in this systematic review. The literature was found to be highly heterogeneous with various cell lines, animal strains, animal ages and chemotherapeutic compounds/ regimens tested, proving difficult to compare outcomes between similar studies or indeed gain any significant insight into which chemotherapeutic regimen caused adverse events. From this analysis we propose a minimum core outcome dataset that could be regarded as a standardised way of reporting results from in vivo experimentation.

Keywords: colorectal cancer; murine animal model; chemotherapy; methodology; core outcome dataset

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1.0 Introduction

Colorectal cancer (CRC) is the third most common carcinoma worldwide with approximately 1.2 million cases and over 600,000 deaths annually (1). Although surgical resection is the mainstay of curative treatment, chemotherapy is used in the majority of patients, either in the adjuvant or neoadjuvant setting, or in an effort to prolong survival in un-resectable disease (2).

Advances in chemotherapy, including new drug combinations, have helped to reduce recurrence rates and prolong survival. Nevertheless, further improvements will only be realised through a greater understanding of the molecular pathogenesis of CRC and the pharmacology of chemotherapeutic and targeted treatments. Animal avatars that replicate human colorectal cancer are an essential component of efforts to achieve these aims.

Although the study of excised human tumour xenotransplants, 3-dimensional in vitro cancer models, and simpler conventional 2-dimensional *in vitro* cell culture systems have led to many insights into CRC biology, these approaches are limited in several critical ways. One example is the heterogeneity observed at a cancer cell and microenvironment level in whole human tumour or biopsy samples, making it difficult to draw inferences about fundamental patterns of cancer cell behavior in response to stimuli. Two- and 3-dimensional cancer cell models address this issue by being typically composed of two to three syngenic cell lines only (e.g. tumour and fibroblasts), however while allowing for greater control of intercellular heterogeneity, these models are not sophisticated enough to permit the full breadth of cellular diversity seen in a real tumour microenvironment (3). Furthermore, the mutational complexity coupled with the chronological and subtle genetic and epigenetic drift of cell lines further limits the use of 2- and 3D cell models (4). For these reasons, controlled and manipulatable *in vivo* studies in genetically defined animal models have been viewed as critical tools necessary to tease out the molecular mechanisms of CRC development and progression.

The drug discovery pipeline attrition rate for oncology compounds is extremely high. Only 5% of anticancer candidate therapies that enter clinical testing are approved by the Food and Drug Administration for clinical practice, suggesting that current murine models do not faithfully reflect human disease (5–7). Reasons for this may be the use of multiple different cell lines, the use of xenograft-based platforms, non-standardized anticancer drug dosing and the lack of clear toxicity and adverse event reporting in pre-clinical anticancer pharmacology studies (5,6). Poor methodological standardisation and reporting in animal studies can result in a failure to translate to the clinical domain (5). Consequently, to maximize bench-to-bedside translation, optimisation, standardisation and consistent reporting of *in vivo* animal experimentation is indispensable.

We therefore conducted a systematic literature search reviewing the methodology and results reporting of preclinical chemotherapeutic drug studies using CRC murine models. From this analysis a minimum core dataset that would be regarded as a standardised way of reporting methodology and results from in vivo experimentation is proposed.

2.0 Methods

2.1 Identification of studies and search strategy

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Two authors (MAW and AR) independently carried out electronic literature searches for study identification and then applied screening. Searches were performed on PubMed (1980 to December 30th, 2015) and EMBASE (1973 to December 30th, 2015) using search terms defined by the reviewers (Supplementary Appendix S1). The "related articles" function was used to broaden the search and all abstracts, studies and citations retrieved were scanned for subject relevance. Complete articles of all potentially relevant publications were retrieved and formally evaluated for inclusion and exclusion criteria independently by the same investigators. The reference list of all retrieved publications was hand searched for additional studies potentially missed by the search strategy (PRISMA diagram - Figure 1).

2.2 Inclusion and exclusion criteria

Only studies that met pre-defined criteria were included in the review process. Pre-clinical colorectal cancer murine models investigating the effects of chemotherapeutic agents were included in this review. Studies were eligible for inclusion if a primary colorectal cancer was established in a mouse by any means (i.e. xenograft, orthotopic, tumour fragment etc.) and treated with known chemotherapeutic and/or biological agents.

Studies that investigated the use of radiotherapy alone, or combined chemoradiation and immunoradiation were excluded. Pre-clinical animal experiments on any other animal species were excluded. Research utilising chemotherapeutic agents to deliberately induce toxicity were excluded. Cancer models investigating colorectal metastasis or peritoneal carcinomatosis not giving any chemotherapy were excluded. Unnamed or experimental anti-cancer drugs, plant based extracts, Chinese herbs or therapeutic sensitising agents e.g. curcumin, cyclo-oxygenase inhibitors or aspirin etc. were also excluded. Pre-clinical models only testing virus delivery systems or other drug delivery systems were excluded. Metronomic drug delivery approaches were excluded. In-vitro cellular experimentation and human clinical trials were excluded. Chemotherapy regimes made up exclusively of monoclonal antibodies were excluded. Chemo-preventative agents and experiments using dietary modulation alone were also excluded. Any disagreement over eligibility of a study was resolved through discussion and review by an additional author until a consensus was reached.

2.3 Data extraction and analysis

Data was extracted by two authors (MAW and AR) using a standardised pro-forma. The study characteristics extracted included: journal, country and year of publication, mouse model type, cell line used, number of cells injected/used, number of mice per group, overall study length, mouse strain, mouse age, chemotherapy regimen, route, adverse events reported, study primary outcome, primary outcome variable observations and its measurement.

3.0 Results

3.1 Literature search and description of studies

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A total of 45 studies were included in this systematic review. Forty-six studies were identified from EMBASE and PUBMED searches (Figure 1), whilst 3 studies were identified from a hand-search of key reference lists.

3.2 Mouse model type and colorectal cell lines

Table 1 summarises all the studies included in this systematic review (9-57), describing the choice of mouse model, CRC cell line, number of cells injected, number of mice used and the overall length of the study protocol.

Forty-three studies utilised a xenograft CRC model, whilst 2 studies utilised pure orthotopic models (2 studies utilised both xenograft and orthotopic models). A wide variety of CRC cell lines were used. The HT-29 CRC cell line (12 studies) was the most frequently used, with patient derived (9 studies) and CT-26 CRC cell lines (7 studies) also frequently used. Thirty-six other CRC cell lines were used, however most of these were used only once. Twenty-two studies reported a specific number of CRC cells injected (ranging from $3x10^3$ to $1x10^7$), 14 studies used tumour fragments and 9 studies did not report the volume or number of cells used to establish CRC in mice.

The number of mice used in each experiment was also a source of wide variability. Although 10 studies reported the exact number of mice in each group, 22 studies reported the total number of animals used in the whole study, 9 reported a range of mice used in each group (ranging from 3-100 animals) and 4 did not report any animal numbers at all. Only 2 studies reported conducting the same experiment in duplicate or triplicate. Length of the studies varied from 12 to 125 days with 3 studies not reporting the experiments' study length. None of the studies detailed an *a priori* sample size calculation.

3.3 Animal characteristics, chemotherapy agents and adverse events

Table 2 describes choice of mouse strain, gender and age at the start of the study protocol, chemotherapy dose and regimen used, chemotherapy administration route and a summary of the reported adverse events.

Eighteen studies used a BALB/c *nu/nu* strain of mouse as a host to establish their CRC model, with 12 studies utilising wild type BALB/c strain. Sixteen other mouse strains were identified. Twenty-eight studies used female mice, 10 used male mice, 2 used a mixture of both, with 5 not reporting the gender of the mice used. A wide variation of animal ages was also used, with 17 studies reporting a specific age (in weeks alive) at the beginning of their experimental protocol, however, 20 studies gave an age range (ranging between 3 to 14 weeks) and 8 studies did not report any animal age.

The chemotherapy regimens and dosages used was a source of extreme variability, with 41 types of chemotherapy drugs used (two studies used a 'FOLFOX like' regimen (16,28) and one used a 'FOLFIRI like' regimen (13)). Across all the included literature only 3 instances were found where an identical chemotherapeutic regimen was utilised (oxaliplatin 10mg/kg in a once only dose either IV or IP (37,42,43), capecitabine 359mg/kg/day orally (37,39) and bevacizumab 5mg/kg twice weekly IP (17,31,34)). Although, 5FU was the most common chemotherapeutic agent used, it was administered using 19 different regimes and only twice at the same formulation

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and regimen (15mg/kg/day IP, 80mg/kg/week IV/IP or 100mg/kg/week IP). Oxaliplatin was used in 14 different regimes, capecitabine in 16, bevacizumab in 8, irinotecan in 8 and cyclophosphamide in 5. Almost all studies (except 1 (50)) reported a dose for the chemotherapeutic agent used. The majority of studies reviewed (27 studies) authors have not referenced the origins of the chemotherapeutic regimens used. Four studies chose chemotherapeutic regimes based on previous preliminary experiments undertaken by the same group; however these data were not published, shown or referenced. Twelve studies chose a chemotherapeutic regimen that was either reported as investigated separately with a dose-escalating regime up to the maximum tolerated dose and/or referenced. One study (15) cited that a similar chemotherapeutic regimen was utilised and was referenced however different doses were used. Another study provided references for some of its chemotherapeutic regimens but for others the authors stated that the regimens were based on previous experiments conducted by the group, however these data were not published or reported (18). Only one study (25) included chemotherapeutic agent calculations based on body surface area.

FOLFOX is the name of a combination chemotherapy treatment used in humans. It is also known as oxaliplatin de Gramont or OxMdG (oxaliplatin modified de Gramont). It is made up of the drugs; FOL – folinic acid (also called leucovorin, FA or calcium folinate), F – fluorouracil (5FU) and OX - oxaliplatin. We found only 2 studies using FOLFOX schedules (16,28). Although both studies used a xenograft model, one was from patient derived CRC cells (16) and the other was a *Msh2*^{LoxP} conditional mouse knock-out used to establish a self-perpetuating intestinal adenocarcinoma model. Both used similar doses of IP folinic acid 10mg/kg, however Lotti et al. used 15mg/kg/dose of 5FU IP and 0.25mg/kg/week of oxaliplatin IP for 2 and 4 weeks respectively, whilst Kucherlapati et al. used 20mg/kg/dose of 5FU IP and 1mg/kg/week of oxaliplatin IP for one week only. Unfortunately both studies do not report any adverse or toxicity events encountered with this regimen.

When evaluating individual FOLFOX components i.e. 5FU, oxaliplatin and leucovorin individually, regimen heterogeneity still existed with 5FU being administered in doses ranging from 4mg/kg/day to 150mg/kg/bolus and leucovorin at 10-20mg/kg/day PO or IP. The commonest 5FU dose was between 15-20mg/kg/day IP and 10mg/kg/day for leucovorin. Stutchbury (25) published a novel all-in-one formulation of 5FU and leucovorin (Fluorodex -FD), where a reduced toxicity profile was reported. They reported a maximum tolerated dose of 5-FU administered between 600 and 675mg/m^2 (200 and 225mg/kg) either as single or fractionated IP bolus doses over 2 weeks i.e. 20-22.5mg/kg/day). This was validated by other studies (Tsukioka (29), Kawabata (42)) administering 5FU as a single agent. Administering 5FU at 15mg/kg/day as an infusion or at 10,40,80mg/kg/week IV alone reported 'tolerable weight changes', however, Komura (12) administered 5FU at lower doses (8.3mg/kg/day with leucovorin at 20mg/kg/day PO) and reported severe weight loss, diarrhoea, mucosal injury and myelosuppression in their animals. Furthermore, it was unsurprising that when 5FU (at near maximum tolerated doses- MTD) was combined with other chemotherapeutic agents, toxicity was encountered (21,46,53) with all studies reporting significant toxic weight loss and animal deaths. When reviewing studies that used much higher doses of 5FU (i.e. 100mg/kg/week IP (14,48); 30mg/kg/day for 5 days IP (52) or 80mg/kg/week IP (44)) no mention of adverse events was found.

Oxaliplatin doses were found to be ranging between 0.25-12mg/kg/week IP. Prewett (33) combined Oxaliplatin 12mg/kg/week IP and Cetuximab 40mg/kg and suggest that this should be considered as the MTD as increasing the dose to 18mg/kg/week IP led to a 60% death rate compared to 0% for a 12mg/kg dose. Similar studies

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reproduced these findings (39,40). Two conflicting studies however were included in this review, with Sawada (34) administering 10 and 15mg/kg/week IV with no deaths or significant weight loss encountered and Nukatsuka (24) administered 4.2 and 8.3mg/kg/week IV encountering unexpected deaths at the higher dose.

The MTD of capecitabine was previously determined in breast cancer-bearing mice as 400 mg/kg per day (q.d.) with a 14/7 schedule (2/3 MTD=267 mg/kg) and 700 mg/kg q.d. with a 7/7 schedule (2/3 MTD=467 mg/kg) (54). Kolinsky administered various doses of capecitabine using different CRC cell line models and did not observe any significant toxicity at 2/3 MTD using either 14/7 or 7/7 schedules alone, in doublet with bevacizumab or irinotecan and triplet with bevacizumab and oxaliplatin. Heijmen (18) also attempted this regimen recently, combining low dose capecitabine 200mg/kg/day PO with oxaliplatin 3 mg/kg weekly and bevacizumab 5 mg/kg twice weekly, however encountering significant toxicity and unexpected deaths in 10 out of 26 mice. Sawada (34) also combined capecitabine (359mg/kg/day PO for 2 weeks) and oxaliplatin (10mg/kg/week) with no significant weight loss reported. Ouchi (36), Nukatsuka (24) and Tsujimoto (26) administered capecitabine at 66% MTD and reported no toxicity either alone or in combination. Cao (37) also established MTDs in nude mice of capecitabine 600mg/kg/day PO for 7 weeks or 400mg/kg PO for 5 days per week for 3 weeks, which replicate similar findings reported above.

Cao (37) also established MTDs for irinotecan at 100mg/kg weekly IV for 3 weeks. This was reduced to 50mg/kg/week IV when combined with capecitabine or 5FU. When, Kanterman (13) attempted to replicate a 'FOLFIRI regimen' using irinotecan 50mg/kg IP combined with 5FU 50mg/kg IP both twice weekly for 3 weeks, they reported a rapid deterioration in animal health, with increased death rates in CRC mice treated with irinotecan alone or 5FU + irinotecan. On bolus dosing (100mg/kg/week IP over 5 weeks), severe loss of weight that necessitated veterinary assistance with fluid therapy was observed. Hare (21) also attempted combining irinotecan (60mg/kg/week for 3 weeks IP) and 5FU (16mg/kg/day for 5 days for 3 weeks IP) and reported increased toxicity (>21% reduction in body weight).

Table 2 summarises the types and number of adverse events that were encountered with the administration of a chemotherapeutic regime. Thirteen studies omitted the reporting of any adverse events. Nine studies reported no adverse events with their selected regimen, however, 7 studies reported severe adverse events almost exclusively linked to animal deaths and 12 studies reported a non-specific description of 'tolerable' or 'intolerable' weight loss. One study (15) reported 'poor condition' following chemotherapy administration.

3.4 Primary outcomes, effects observed and measurements used

Table 3 briefly summaries the individual studies primary outcomes, key findings with the effects observed on the CRC model and the techniques/ methodology utilised to interrogate the effects observed. The majority of the studies (38 studies) had a study primary outcome directly related to the evaluation of chemotherapeutic effectiveness on CRC. Seven studies investigated chemotherapeutic effectiveness in relation to specific molecular targets (4 studies) and drug metabolism (3 studies). Most studies utilised a measure of tumour volume or size as their primary outcome measure.

4.0 Discussion

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Rodents and humans share many biological functions, hence the attractiveness of rodent cancer models to test chemotherapeutic toxicity and tumour responses, as equivalent human experimentation is time consuming, costly and could jeopardise patients well being.

We have performed a systematic review of the methodology and results outcome reporting from preclinical chemotherapeutic drug studies using CRC murine models. We have summarised 45 studies giving details of chemotherapeutic usage, dosage, animal model choice, toxicity and adverse effects encountered. The literature was found to be highly heterogeneous with various cell lines, animal strains, animal ages and chemotherapeutic compounds/ regimens tested, making any comparison of significant outcomes between similar studies difficult to achieve, and confounding reproducibility of findings, both of whom are important considerations for successful clinical translation. Gaining any significant insight into which chemotherapeutic regimen caused an adverse event is very challenging. Consequently, using this body of evidence to aid future study design and validate key components of chemotherapeutic delivery and response in a pre-clinical mouse model would be challenging due to the unclear reporting of methodology and outcome measures.

We know that animal models are critical for the development of novel chemotherapeutics; however they have been minimally successful in decreasing the age-adjusted death rate from cancer when compared with for example animal modeling of cardiac disease. It is important to review the approaches by with tumour models especially xenograft models are established, cancer drug testing is conducted and new anti-cancer therapeutics promoted from bench-to-bedside (55). Unfortunately, many variables exist when conducting tumor model experiments that may impact on outcome, including: site of tumor implantation, growth properties of the xenograft, size when treatment is initiated, agent formulation, scheduling, route of administration, dose and the selected endpoint for assessing activity. There are also multiple potential confounding factors when trying to model multi-agent cytotoxic chemotherapies in animal models. For example platinum based chemotherapy can appear more nephrotoxic in mice, as adequate hydration is not easily achieved. Furthermore rodents have much higher levels of circulating thymidine and so thymidine synthase inhibition can be circumvented by thymidine salvage resulting in a different therapeutic index. The xenograft model remains of value in current preclinical cancer drug development, especially if studies give due consideration to the above variables and are based on sound mechanistic (e.g. status of the selected target in the chosen model) and pharmacological (e.g. use of formulated agent) principles. Human tumour xenografts are also particularly useful in determining pharmacodynamic markers of response for subsequent clinical application. Nevertheless, it needs to be kept in mind that the use of xenografts is relatively time-consuming and expensive, and there are instances where the model is inappropriate as a likely predictor of clinical outcome. In the near future xenograft models may be replaced or complemented by patient-specific organoid models which may eliminate animal use, are amenable to high throughput drug screening, closely recapitulate several properties of original tumours and allow detection of gene-drug interactions (56). The use of xenograft murine models in this review was almost exclusive with 48 out of 45 included studies still utilising this model for chemotherapeutic effectiveness studies.

Despite new insight into the pathogenesis and development of cancer, most novel therapies fail upon reaching Phase III clinical trials. This occurs even though millions of dollars are spent on target validation and drug optimization in preclinical models.

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When evaluating our approach to target discovery, we should consider if our current, powerful genomic technologies are being used on model systems that have poor clinical predictive power. For example, patient-derived xenograft (PDX) tumor models have emerged as a new approach to evaluate the effects of cancer drugs on patients' personalized tumor grafts enabling to select the best treatment for the cancer patient and providing a new tool for oncology drug developers (57,58). With novel targeted agents therefore, it could be argued that these models now potentially have a clearer role in helping to develop patient stratification hypotheses and biomarker readouts that are easily translatable from bench-to-bedside. Furthermore, identification of biomarkers that provide rapid and accessible readouts of drug exposure, activity, toxicity or efficacy is becoming increasingly important in the clinical development of novel molecularly targeted therapeutics. Surrogate endpoints can be applied in the assessment of biological activity or clinical responses and perhaps in selection of patients most likely to respond to therapy. Methodologies that incorporate an analysis of candidate biomarkers using tumour or other tissues in preclinical animal studies need further evaluation.

The heterogeneity of study design and poor quality of reporting in the reviewed literature highlights the importance of establishing minimum and consistent criteria for the conduct and reporting of murine experiments. For example study heterogeneity especially when considering the administration of a human like ‘FOLFOX’ or ‘FOLFIRI’ regimes makes the replication and validation of any chemotherapeutic regimes very difficult. This is especially important in facilitating appropriate comparisons between studies utilising similar chemotherapeutic compounds to interrogate different hypotheses. Furthermore, the reported inconsistencies of various tumour models testing new chemotherapeutic regimens make the introduction of a minimum core dataset even more urgent. With many reports showing widely varying responsiveness and adverse events to commonly used chemotherapeutic agents, factors like tumour cell line selection, period of cell inoculation, animal weight, gender and strain, chemotherapy dose, scheduling and route of administration need particular attention and clarity of reporting during the design of such preclinical experiments (54,59).

These issues could be addressed through the development and use of an agreed standardized collection of outcomes, known as a minimum core outcome dataset or core outcome set (COS) (60). These sets do not imply that outcomes in a particular trial or experiment should be restricted to those in the COS. In January 2010, the Core Outcome Measures in Effectiveness Trials (COMET) initiative, launched aims to facilitate the development and application of COS in human clinical trials. COS in human clinical trials are now endorsed and supported by the GRADE (Grading of Recommendations Assessment, Development and Evaluation) group (http://www.gradeworkinggroup.org), by Cochrane Reviews of the effects of healthcare interventions (61,62) and by the World Health Organization (WHO) in developing guideline recommendations (63). Minimum core outcome datasets are disease or population specific, but not trial specific; and the concept might be easily translatable to preclinical animal experimentation (64). Selection of appropriate outcomes or domains is crucial when designing clinical or pre-clinical trials, however their standardized reporting makes comparison between studies (even when different interventions are used) easy. Indeed in a pre-clinical trial setting this approach would enhance the value of evidence synthesis by reducing heterogeneity in reported outcomes between experiments and reducing the risk of outcome reporting bias, since reports will always include the presentation of the minimum core outcome dataset. Moreover, statistical power would be increased because fewer experiments would have to be omitted from meta-analyses.

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The introduction of a minimum core outcomes dataset would serve as a structured framework to guide future trial design and robust reporting. We suggest that research groups designing and reporting on *in vivo* animal experimentation involving cancer and chemotherapy should report their data using 3 main outcome headlines: animal characteristics, cancer and chemotherapy characteristics, and adverse events (summarised in table 4). Animal characteristics would include the reporting of core outcomes including: number of animals used per group (with no ranges), animal strain, gender, and age (in weeks) at initial treatment. Cancer and chemotherapy characteristics would include: animal model description (xenograft, orthotopic etc.), cancer cell line/ tumour fragment with its origin, number of cancer cells injected (if applicable), chemotherapeutic regime with the name of the compound, dose (in mg/kg or corrected for body surface area), timing of dose (i.e. per day, per week, bolus, twice daily etc.), overall number of cycles, overall length of study from initial chemotherapy treatment, and the route of drug administration. It would also be desirable to report and reference the origins of the selected chemotherapeutic regime, i.e. if this was tested in a feasibility/pilot/phase 1 setting, already tested MTDs or already reported by others. Finally, adverse events encountered during the experiment should include: number of events reported, severity, number of unexpected deaths, a definition of acceptable weight loss (e.g. >15% of initial body weight) before culling or rescue treatment, and any extraordinary measures of support i.e. veterinary assistance, subcutaneous fluid administration or extra warming.

In conclusion, discordance on many fundamental methodological and study design aspects, most notably reporting heterogeneity exists between studies. Studies lack consistent reporting of important methodological and result core outcomes. This makes studies very difficult to interpret, challenging to meta-analyse and construct robust future experimental design. The authors propose and recommended the use of a minimum core outcome dataset that may provide a framework for standardised and transparent reporting for in vivo animal experimentation. The proposed dataset would need further validation using a consensus-based method such as a Delphi method (65), which is generally considered to be an appropriate methodology to determine the extent to which experts in the field agree on such outcomes. It is hoped the proposed minimum core outcome dataset would greatly benefit and improve evidence synthesis, reduce reporting bias and enhance future experimental design.

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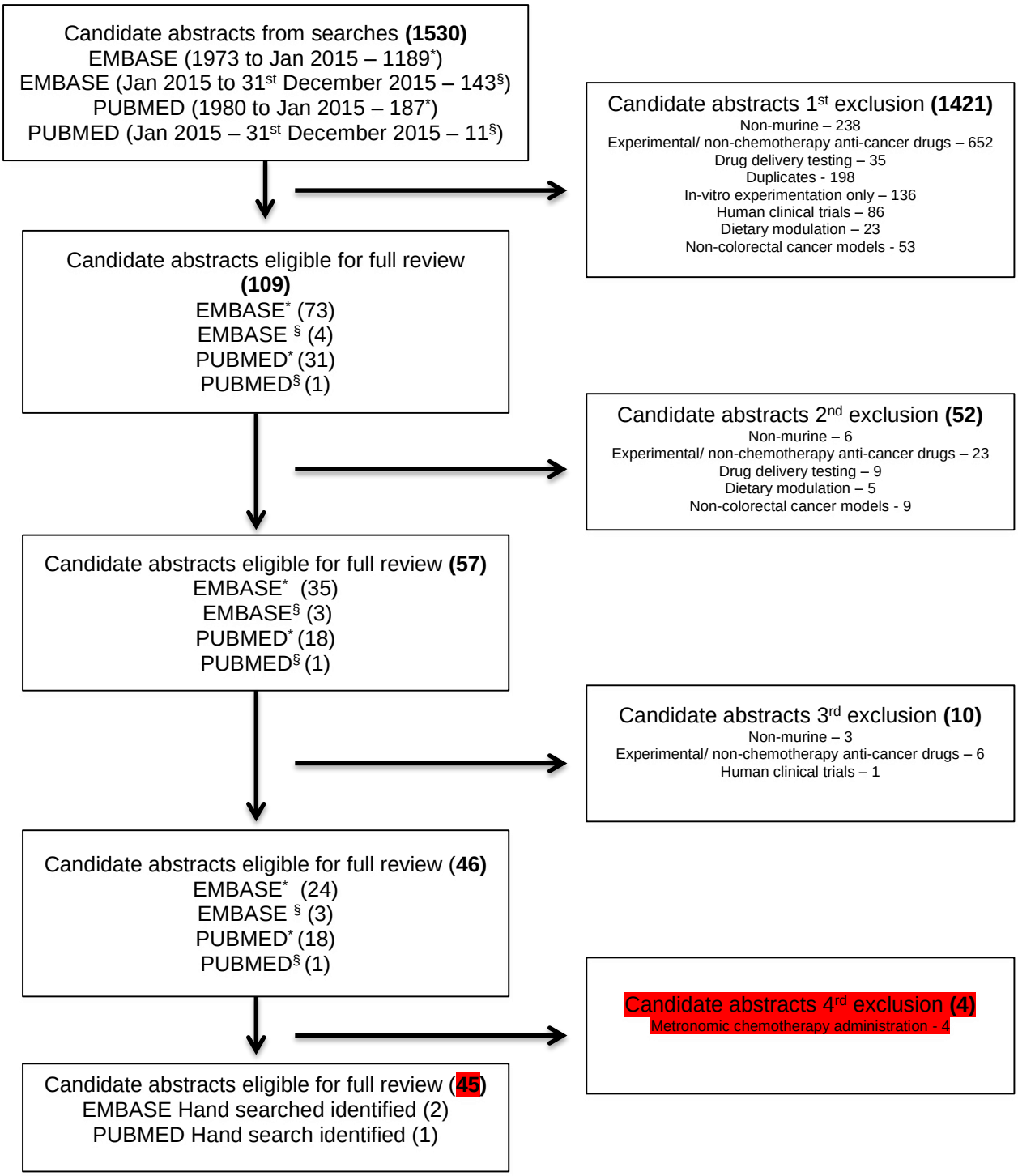
Figure 1 – PRISMA flow diagram for inclusion, screening and exclusion of abstracts and full text articles



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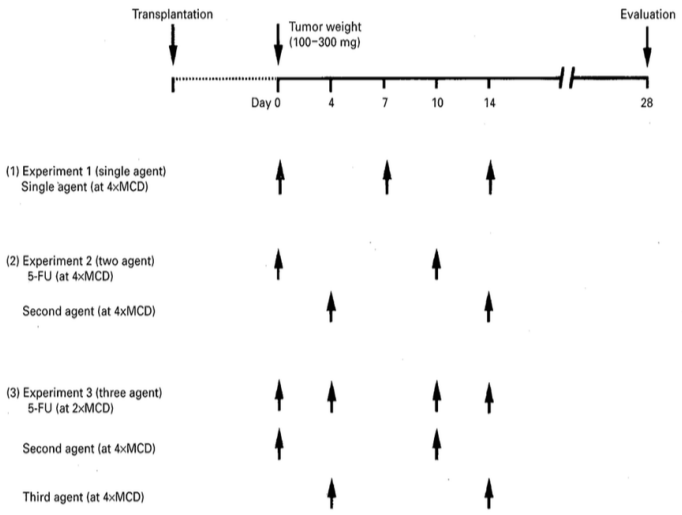

Figure 1. Protocol for the drug administration. Drugs were given when the tumor had grown to 100–300 mm^3, usually at 2–3 weeks after the transplantation. Tumor-bearing mice were randomized into groups of four to six mice each, i.e. the treated and control groups. Each experiment was repeated twice and the results were calculated from the accumulated data (each group included eight to 12 mice in total). In experiment 1, each single agent was administered at 4 × MCD (Table 1) weekly on days 0, 7 and 14. In experiment 2, 5-FU was administered on days 0 and 10 at 4 × MCD, and another agent was administered at 4 × MCD on days 4 and 14. In experiment 3, 5-FU was given as basic agent at half dose (i.e. 2 × MCD) on days 0, 4, 10 and 14 (total dose of 5-FU was the same as in experiment 2) and other two agents were alternatively administered at 4 × MCD on days 0, 4, 10 and 14. The efficacy of each regimen was assessed on day 28 after the start of treatment; the duration from the termination of the treatment to the day of evaluation was 14 days. The mice in the control group were given 1 ml of saline.

Figure 2 – Protocol for chemotherapy administration in Kawabata et al (42)

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Table 1 – A summary of all the studies included describing the choice of mouse model, CRC cell line, number of cells injected, number of mice used and the overall length of the study protocol.

Author	Year	Country	Mouse Model	Cell line	No cells injected/used	Number of mice used	Length of study (days)
Napolitano (9)	2015	Italy	Xenograft and Orthotopic	GEO-CR SW48-CR HCT 116 HCT15	3.5×10^6 3.5×10^6 $2x10^6$ $2x10^6$	10 per group in xenograft model and 7 per group in orthotopic model	Up to 119
Lee (10)	2015	Taiwan	Xenograft	HT29	1×10^4	15 in each group, 5 in control group	21
Tongu (11)	2015	USA	Xenograft	CT26	5×10^5	6-7 in each group	38
Komura (12)	2015	Japan	Xenograft	HT-29, DLD-1	Not reported	18	28
Kanterman (13)	2014	Israel	Xenograft	Patient derived	N/A	24 (6 per group) repeated in triplicate	77
Chao (14)	2014	Taiwan	Xenograft	CT-26	1×10^6	68	21
Gaur (15)	2014	Taiwan	Xenograft	HT-29	3×10^5	40	21
Lotti (16)	2013	USA	Xenograft	Patient derived	3×10^3	20 (3-6 per group)	30
Jure-Kunkel (17)	2013	USA	Xenograft	CT-26	Not reported	8-12 per group	40
Heijmen (18)	2013	Netherlands	Xenograft	LS174T CRC	$1x10^6$	25	12
Abou-Elkacem (19)	2013	Germany	Orthotopic	CT26	$2x10^6$	46	14
Kendrew (20)	2013	UK	Xenograft	LoVo LS174T	$1x10^4$ $1x10^6$	9-15 per group	Up to 30
Hare (21)	2013	Canada	Xenograft	HT29	$5x10^6$	24 (6 per group)	56
Wehler (22)	2013	Germany	Xenograft	HT29	1×10^7	20	28
Jin (23)	2012	China	Xenograft	Patient derived	N/A	10 per group (4 groups)	30

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Nukatsuka (24)	2012	Japan	Xenograft	COL-1, KM12C/ 5-FU	8 mm^3 volume was injected	7-9 / group (3 groups)	22
Stutchbury (25)	2011	Australia	Xenograft	HCT-116, HT-29	Not reported	Not reported	28
Tsujimoto (26)	2010	Japan	Xenograft	COLO-201 COLO-320DM WiDr COLO-205 HCT-15 DLD-1 LoVo COL-1 Co-3 KM12C/5-FU	Tumour fragments	6 per group	30
Li (27)	2010	Japan	Xenograft	DLD-1	5×10^6	20	35
Kucherlapati (28)	2010	USA	Xenograft	*Msh2*^{LoxP}	Not reported	17	14
Tsukioka (29)	2009	Japan	Xenograft	COL-1 KM12C KM20C	Not reported	Up to 10 mice in each group	14
Kolinsky (30)	2009	USA	Xenograft	HT29 COLO-205	$3x10^6$ $5x10^6$	80 (10 per group)	Up to 100
Yanagisawa (31)	2009	Japan	Xenograft	COL-16-JCK, COLO 205, CXF280	COL-16-JCK and CXF280- Tumour fragments COLO 205 - $5x10^6$ or $8.8x10^6$	24	41
Kolinsky (32)	2009	USA	Xenograft	HT-29	3×10^6	40	125
Prewett (33)	2007	USA	Xenograft	Oxaliplatin-resistant HT29-OxR, KM12-OxR	5×10^6	48	41
Sawada (34)	2007	Japan	Xenograft	CXF280	Tumour fragment	6 per group and 8	Up to 61

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				COL-05-JCK		per group in the COL-05-JCK line	
Tabernero (35)	2007	Spain	Xenograft	GEO	$1x10^7$	10 per group	56
F-Ouchi (36)	2006	Japan	Xenograft	LoVo, HT-29	$5×10^6$	11	15
Cao (37)	2005	USA	Xenograft	HCT-8, HT-29	50 mg tumor fragment	15-20 per group (9 groups)	32
Kamm (38)	2003	Netherlands	Xenograft	C26-B C26-10	Tumour fragment	11 and 15	28
Tortora (39)	2002	Italy	Xenograft	GEO colon	$1x10^7$	30	70
Louvet (40)	2000	France	Xenograft	HT-29	$1×10^7$	100	28
Guichard (41)	1998	France	Orthotopic	C26	$2×10^6$ + serial IP transplantation of malignant ascites every week (10^6)	Not reported	39
Kawabata (42)	1997	Japan	Xenograft	CC-KK, RC-TK	Not reported	4-6 per group (3 groups)	28
Jan (43)	1996	Netherlands	Xenograft	Colon 26-A, Colon 26-B, Colon 26-10, Colon 38	Tumour fragments 1-5 mm^3	Not reported	42
Van Laar (44)	1996	USA	Xenograft	Colon 26-B	Tumour fragments	36	27
Wilmanns (45)	1992	USA	Xenograft, Orthotopic	CT-26	2.5×10^4 (Xenograft) 2×10^4 (Spleen) 5×10^4 (Caecum)	3 in each group repeated 4-6 times	22
Giuliani (46)	1981	USA	Xenograft	Patient derived	Tumour fragment	6-10 per tumour group	Up to 70
Houghton (47)	1981	USA	Xenograft	Patient derived	Tumour fragment	Not reported	Not reported
Giuliani (48)	1981	USA	Xenograft	Patient derived	Tumour fragment	6-10 per tumour	Up to 35
Schmitz (49)	1980	Germany	Xenograft	Patient derived	Tumour fragment	15	Up to 70
Warenius (50)	1980	UK	Xenograft	HT29	$1x10^6$	59	44

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Houghton (51)	1978	UK	Xenograft	HxBR (rectum) HxAC4 (caecum) HxHC1 (Ascending colon) HxGC3 (transverse colon) HxVRC5 (caecum) HxELC2 (Caecum)	Tumour fragment	4 in each group	Not reported
Nowak (52)	1978	UK	Xenograft	Patient derived	Tumour fragment	Between 6-53	Up to 60
Corbett (53)	1977	USA	Xenograft	Patient derived	Tumour fragments	10 per group	Not reported

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Table 2 – A summary of all the studies included describing the choice of mouse strain, gender and age at the start of the study protocol, chemotherapy dose and regimen used, chemotherapy administration route and a summary of the reported adverse events.

Reference number	**Mouse strain**	**Gender**	**Mouse age**	**Chemotherapy doses/regime**	**Route**	**Reported Adverse Events**
(9)	BALB/c *nu/nu*	Female	4-6 weeks	Group 1 – Control Group 2 - Cetuximab twice weekly 1 mg/dose for 3 weeks Group 3 - Regorafenib daily 10 mg/kg for 3 weeks. Group 4 - Combination of regorafenib and cetuximab	IP, PO IP PO IP, PO	Single or combined treatments were tolerate with no side effects or weight loss in the xenograft model, however in the orthotopic model significant weight loss occurred in single agent treatment groups
(10)	SCID NoD	Male	8 weeks	Group 1 - Sorafenib 80mg/kg and Lapatinib 60mg/kg for 10 days. Group 2 - Control groups Lapatinib 1mg/kg daily for 10 days	PO PO	Following Sorafenib and Lapatinib treatment, the body weights decreased by approximately 2–3 g in four mice and 4 g in one mouse, and no such loss in body weight was observed in five mice. In mice treated with Lapatinib, the body weight decreased by approximately 0.2 g in one mouse, and no such decrease occurred in four mice. Because the decrease in body weight was also observed in the control mice, the decreased body weight may have resulted from exsanguination.

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(11)	BALB⁄c and BALB⁄c *nu/nu*	Female	6-7 weeks	Group 1 – Cyclophosphamide 50mg/kg and Gemcitabine 50mg/kg on day 10 and 18 Group 2 - Control	IP IP	Not reported
(12)	BALB/c *nu/nu*	Male	4 weeks	Group 1 - Control Group 2 – 5FU/Leucovorin, 8.3 and 20mg/kg/day, on days 1–14 and 0.5 % HPMC on days 15–28 [+] Group 3 5FU/ Leucovorin 8.3 and 20mg/kg/day, or 0.5 % HPMC on alternate days for 28 days [+]	PO PO PO	No treatment-related death was observed in any of the groups. Severe weight loss and diarrhoea were observed only in the daily group Mucosal injury and myelosuppression were more severe in the daily group
(13)	BALB/C and C57BL/6	Female	6-8 weeks	Group 1 – Control Group 2 - 5FU 50mg/kg Group 3 - Irinotecan 50mg/kg Group 4 - FOLFIRI “like” – 5FU 50mg/kg, Folinic acid and Irinotecan 50mg/kg Twice a week in a 3-day interval for 3 weeks	IP IP IP IP	The daily recorded vitality and survival of the mice show a rapid deterioration, with increased death rates in CRC mice treated with Irinotecan or 5FU + Irinotecan, as compared with the 5FU, or Irinotecan-treated myeloid-derived suppressor cells-depleted CRC mice, or even to untreated CRC mice

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(14)	BALB/c	Not reported	6 week	Group 1 - Control - reverse osmosis water daily Group 2 – 5-FU, 100 mg/kg/ week (2 cycles) Group 3 – Rapamycin, 2 mg/kg/day for 2 weeks Group 4 –combined treatments for 2 weeks	PO IP PO PO, IP	Not reported
(15)	BALB/cAnN. Cg-*Foxn1*^{nu}/ CrlNarl	Female	5 weeks	Group 1 - Control Group 2 – Oxaliplatin, 6.7 mg/kg weekly for 3 weeks Group 3 – Dovitinib, 60 mg/kg every 2 days for 3 weeks Group 4 - Combined treatments $^{+}$ Similar treatment to sited paper but not same doses	 IV PO PO, IV	Combination did not show any gross signs of toxicity and/or possible adverse side effects as measured by body weight and diet consumption.
(16)	NSG	Female	6 weeks	Group 1 - Control (2 mg/kg antibody 5 times a week for 2 weeks and then once a week for 2 weeks) Group 2 - FOLFOX alone (Oxaliplatin: 0.25 mg/kg once a week for 4 weeks; 5-FU, 15 mg/kg 5 times a week for 2 weeks)	IP IP	Not reported

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				Group 3 - IL-17A antibody alone (2 mg/kg 5 times a week for 2 weeks and then once a week for 2 weeks) Group 4 - Combination of the IL-17 antibody and FOLFOX chemotherapy.	IP IP	
(17)	BALB/c	Female	8-12 weeks	Group 1 - Ipilimumab 20mg/kg – day 5,9,13* Ixabepilone 8mg/kg – day 4,8,12* Ipilimumab and Ixabepilone - day 5,9,13 and day 4,8,12* Paclitaxel 24mg/kg – day 4,8,12 * Paclitaxel and Ipilimumab – day 4,8,12 and day 5,9,13* Group 2 – Ipilimumab – day 9,13,17* Etoposide 50mg/kg – day 8,15,22* Ipilimumab and Etoposide day 9,13,17 and day 8,15,22* Group 3 – Ipilimumab – day 9,13,17* Gemcitabine 120mg/kg – day 8,12,16* Ipilimumab and Gemcitabine –	IP IP IP IP IP IP IV IP and IV IP IP IP	Following administration of Ipilimumab and each chemotherapeutic agent the authors did not observe weight loss above the levels observed with the chemotherapeutic agent alone.

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				day 8,12,16 and day 9,13,17*		
(18)	BALB/c	Female	6-8 weeks	Group 1 – Control Group 2 - Oxaliplatin 3 mg/kg weekly, Capecitabine* 200 mg/kg daily and Bevacizumab 5 mg/kg twice a week +	IP IP/Oral	10 out of 26 mice had to be euthanized due to “poor condition”
(19)	CD1 nude	Female	6-8 weeks	Group 1 - Regorafenib 30mg/g for 10 days Group 2 – Control Group 3 - DC101 VEGFR2-anitbody 34mg/kg every 3rd day for 10 days	PO PO IP	Both DC101 and Regorafenib were well tolerated with no significant weight loss
(20)	Nude (Swiss *nu/nu*)	Female	6 weeks	Group 1 - Cediranib 3mg/kg/day daily for 3 days* Group 2 – Cediranib + Irinotecan 25mg/kg weekly Group 3 – Cediranib +5FU 50mg/kg weekly Group 4 – Cediranib + Oxaliplatin 7.5mg/kg weekly (All for 3 weeks)	PO PO/IV PO/IV PO/IV	Not reported
(21)	Rag2-M	Female	6-10 weeks	Group 1 – Control Group 2 - 5FU 16mg/kg (daily for 5 days x 3 weeks) Group 3 - Irinotecan 60mg/kg (weekly for 3 weeks)* Group 4 - Liposomal Irinotecan 40 or 60 mg/kg (weekly for 3 weeks)* Group 5 – Irinotecan +5FU	IP IP IP IV IP	5FU and IV Irinotecan showed increased toxicity (>21% reduction in body weight) when compared to liposomal Irinotecan

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				Group 6 – Liposomal Irinotecan + 5FU*	IV	
(22)	nod-SCID	Female	7-8 week	Group 1 - 5 days/week; 25% cremophor in 0.9% saline (4 weeks) Group 2 – Sorafenib, 5 days per week; 0.12 mg/dose solved in 25 % cremophor; 30 mg/kg/ week (4 weeks) + Group 3 – 5-FU, three times a week; 0.18 mg/ dose solved in 25 % cremophor; 25 mg/kg/week (4 weeks)+ Group 4 – Combined treatments (4 weeks)	IP IP IP IP	Combination might only add adverse events
(23)	BALB/c nude	Female	Not reported	Group 1 –Control Group 2 - Bevacizumab 10mg/kg twice weekly for 3 weeks Group 3 - Cetuximab 10mg/kg twice weekly for 3 weeks Group 4 – Cetuximab and Bevacizumab	IP IV IP IP/IV	Not reported
(24)	BALB/c nude	Male	4 week	S-1 was prepared by mixing tegafur, gimestat, and potassium oxonate at a molar ratio of 1:0.4:1 in 0.5% HPMC.		5/7 mice treated with Oxaliplatin at a dose of 8.3 mg/kg on day 1 had died before day 15. Day 1 administration was thought to be toxic in this model.

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				Group 1 - S-1 - 6.9 mg/kg once daily, for 14 days. Group 2- Capecitabine 360 mg/kg daily, for 14 days. Group 3- Oxaliplatin 8.3 mg/kg on day 1 alone or day 8 alone. Group 4 - Combination treatment with Oxaliplatin 4.2 mg/kg on days 1 and 8 with S-1 6.9 mg/kg daily for 14 days *	PO PO IV PO, IV	Combination treatment 5/7 died
(25)	Balb/c *nu/nu*	Female	Not reported	Group 1 – Control phosphate buffered saline Group 2 - Single or multiple (fractionated) doses of the FD formulation of 5-FU (15 mg/ml) and Leucovorin (1 mg/ml) + Group 3 - sequential 5-FU:Leucovorin treatment - with Leucovorin being administered immediately before 5-FU +	IP IP IP	A dose-dependent increase in toxicity was only observed in animals administered with FD or 5-FU:Leucovorin (15:1) as either a single IP dose of greater than 600 mg/m^2 or as a fractionated IP dose of greater than 120mg/m^2 x 5 (within 14 days). The maximum tolerated dose of 5-FU administered either as 5-FU:Leucovorin or within FD lies between 600 and 675 mg/m^2 of 5-FU given either as single or fractionated IP doses over 2 weeks.
(26)	BALB/c *nu/nu*	Male	Not reported	Group 1 - 5FU Prodrug (UFT) 20mg/kg/day 14/7+ Group 2 – UFT and Leucovorin 10mg/kg/day 14/7+ Group 3 - Capecitabine 450mg/kg/day 14/7+	PO PO PO	Since the body weight change in each drug group was higher than -20% during the experimental period, the toxicity of each drug was within the tolerated range and did not interfere with assessment of the antitumor effect (data not shown).

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(27)	BALB/c, *nu/nu*	Female	5 weeks	Group 1 – Control Group 2 - 5- FU 30 mg/kg every 5th day a total of 5 cycles Group 3 - 3 methylade- nine (3-MA) 24 mg/kg every 5th day a total of 5 cycles Group 4 – both 5- FU 30 mg/kg and 3-MA every 5th day a total of 5 cycles	IP IP IP IP	Not reported
(28)	C57BL/6J Msh2 knockout (VCMsh2$^{LoxP/null}$ and VCMsh2$^{LoxP/G674D}$)	Female	Not reported	Group 1 - Cisplatin 20 mg/kg, five times every second day with a total dose of 100 mg/kg body weight Group 2 - FOLFOX - 5-fluorouracil/leucovorin, five sequential days 20 mg/kg and 10 mg/kg respectively; Oxaliplatin 1 mg/kg once Group 3 – Control group - Phosphate Buffered Saline (PBS) five times every second day.	IP IP IP	Not reported
(29)	BALB/c *nu/nu*	Male	4-5 weeks	Group 1 – Control Group 2 - Oral pro-drug (S1) 6.9 or 8.3mg/kg/day Group 3 - Oral pro-drug (S1) 6.9 or 8.3mg/kg/day and	 PO PO	“Body weight change was tolerable”

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				Leucovorin 10 or 20mg/kg/day Group 4 - 5FU 15mg/kg/day Group 5 – 5FU and Leucovorin	Infusion PO/Infusion	
(30)	Nude	Female	13-14 weeks	<u>HT29 Model</u> Group 1 to 6 - Capecitabine 400mg/kg/day (qd) and ⅔ MTD 267mg/kg (qd) for 14/7 schedule. 700mg/kg/day (qd) and ⅔ MTD 467mg/kg (qd) for 7/7 schedule Group 7 to 12- Capecitabine and Bevacizumab 5mg/kg twice weekly Group 13 to 18 Capecitabine, Bevacizumab and Oxaliplatin 6.7mg/kg weekly for 3 weeks <u>COLO-205</u> Group 19 to 21 - Capecitabine 360mg/kg (qd) daily for 6 days and Bevacizumab 2.5mg/kg twice weekly	PO PO/IP PO/IP PO/IP	No evidence of toxicity with any of the Capecitabine 7/7 or 14/7 monotherapy or combination regimens. Data for average percentage weight change showed no meaningful changes during the treatment course and no significant difference between the treatment groups was reported (data not shown)
(31)	BALB/c *nu/nu*	Male	5 weeks	Group 1 - Bevacizumab - 1.2, 2.5 and 4.0 mg/kg administered	IP	Not reported

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				twice a week for 3 weeks Group 2 - Bevacizumab - 4 mg/kg twice a week for 3 weeks and capecitabine 359 mg/kg daily for 14 days. Group 3 - Capecitabine - 180mg/kg daily for 14 days. Oxaliplatin - 5 mg/kg on Day 1 and 4 mg/kg Bevacizumab twice a week for 3 weeks.	IP, PO IP, PO, IV	
(32)	Athymic nude	Not reported	3 to 14 weeks	Group 1 – Capecitabine 14/7 at 267mg/kg daily + irinotecan 40 mg/kg every 4 days for 5 doses ("capecitabine 14/7 doublet")[+] Group 2 – Capecitabine* 7/7 at 467mg/kg daily + irinotecan 40 mg/kg every 4 days for 5 doses ("capecitabine 7/7 doublet")[+] Group 3 – Capecitabine* 14/7 at 267mg/kg daily + irinotecan 40 mg/kg every 4 days for 5 doses + bevacizumab 5 mg/kg 2x/week ("capecitabine 14/7 triplet")[+] Group 4 – Capecitabine* 7/7 at 467 mg/kg daily + irinotecan 40 mg/kg every 4 days for 5 doses	PO, IP PO, IP PO, IP PO, IP	There was no toxicity with any of the capecitabine 7/7 and 14/7 doublet and triplet combination regimens tested. Data for average percentage weight change showed no significant changes during the treatment course and no significant differences between the treatment groups.

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				+ bevacizumab 5 mg/kg 2x/week ("capecitabine 7/7 triplet")+ Group 5 – Control. (Irinotecan days 1, 5, 9, 13, 17)		
(33)	*Nu/nu*	Female	7-8 weeks	Group 1 - Control Group 2 - Cetuximab 40mg/kg Group 3 - Oxaliplatin 12 mg/kg, every 7 days Group 4 - Combination of Cetuximab 40mg/kg and Oxaliplatin 12 mg/kg, every 7 days	IP IP IP IP	The dose of oxaliplatin used is the maximum tolerated dose with this treatment schedule. Increasing the oxaliplatin dose to 18 mg/kg in the HT-29 xenograft model resulted in 13% body weight loss by day 25 of treatment and 60% lethality by day 29 of treatment, compared with 8% and 0%, respectively, for the 12 mg/kg dosing regimen.
(34)	BALB/c *nu/nu*	Not reported	4-5 weeks	Group 1 – Control Group 2 - Capecitabine 359mg/kg daily for 14 days Group 3 – Oxaliplatin 10mg/kg once only Group 4 – Oxaliplatin 15mg/kg once only Group 5 – Capecitabine 539 mg/kg Group 6 – Oxaliplatin 10mg/kg and Capecitabine 359mg/kg	 PO IV IV PO IV/PO	No weight loss reported
(35)	BALB/c *nu*$^+$/*nu*$^+$	Female	4-6 weeks	Group 1 – Control Group 2 - Oxaliplatin 15mg/kg weekly for 3 weeks	 IP	Not reported

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				Group 3 - Cetuximab 1mg/mouse twice weekly for 3 weeks Group 4 – Oxaliplatin and Cetuximab combination	IP IP	
(36)	BALB/c *nu/nu*	Male and Female	4-6 weeks	<u>Experiment 1 - LoVo bearing mice</u> Group 1 - Erlotinib at doses of 50, 75, 100 or 125 mg/kg once daily for 2 weeks. Group 2 – Control <u>Experiment 2 - LoVo, HT-29 bearing mice</u> Group 1 Erlotinib 100 mg/kg once daily for 2 weeks Group 2 - Control <u>Experiment 3 - LoVo bearing mice</u> Group 1 - Erlotinib 100 mg/kg [80% MTD]$^{+}$ Group 2 - Capecitabine 359 mg/kg (66% MTD)$^{+}$ Group 3 - Combination of erlotinib 100 mg/kg and capecitabine 359 mg/ kg or combined vehicle control, once daily for 2 weeks^{+} Group 4 – Control	 PO PO PO PO PO	Erlotinib in combination with capecitabine was not associated with significantly increased toxicity compared with single-agent therapy and there was no mean body weight loss greater than 20% of pretreatment body weight. Erlotinib 250 mg/kg/day was lethal in this model (data not shown), indicating that the MTD for erlotinib is 125 mg/kg/day
(37)	Nude	Female	8-12	Group 1 - Capecitabine	PO	No increased toxicity reported with

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			weeks	Group 2 - Irinotecan Group 3 - Irinotecan in combination with capecitabine 2 different schedules: (1) Capecitabine 600mg/kg once a day for 7 days and a single dose of irinotecan 50 mg/kg (2) Capecitabine 400mg/kg orally 5 days a week for 3 weeks and irinotecan 50 mg/kg once a week for 3 weeks	IV PO, IV	combination treatments
(38)	BALB/c	Female	8-12 weeks	Group 1 – Control Group 2 - 5FU 150mg/kg bolus	IP IP	Mean percentage weight loss between day 0-28 was 6% for C26-B and 12% in the other groups
(39)	BALB/c *nu*$^{+}$/*nu*$^{+}$	Female	5-6 week	Group 1 - Oxaliplatin either: 10mg/kg on day 1 every week for 4 weeks or 15mg/kg on day 1 every 2 weeks $^{+}$ Group 2 – Topotecan either: 2mg/kg on day 1 every week for 4 weeks or 2 mg/kg on day 1 and 2mg/kg every 2 weeks or 0.5mg/kg on days 1-4 every 2 weeks $^{+}$ Group 3 – Oxaliplatin 10mg/kg on day 1 and topotecan 0.5mg/kg on day 2-5. Both administered every 2 weeks $^{+}$ Group 4 – control	IP IP IP IP	Oxapliplatin alone was ineffective in inhibiting tumor growth since by day 35 all oxaliplatin treated mice along with all mice in the control group were dead. No signs of acute or delayed toxicity were observed in treated mice.

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				Each treatment was completed for a total of 3 cycles.		
(40)	BALB/c nude *nu*$^{+}$/*nu*$^{+}$	Female	6 weeks	Group 1 - Control Group 2 – Oxaliplatin 10 mg/kg given on day 1 Group 3 – UFT 20 mg/kg/day from days 1 to 28, folinic acid 4 mg/kg/day from days 1 to 28 Group 4 – Oxaliplatin 10 mg/kg given on day 1, UFT 20 mg/kg/day from days 1 to 28 and folinic acid 4 mg/kg/day from days 1 to 28	PO IP PO PO, IP	No adverse effect was observed in any of the three treatment groups: all the mice were alive at day 28 and mean body weight was similar in the four experimental groups.
(41)	BALB/c mice	Female	5 week	Mice treatment occurred on days 2, 6, and 10 after cell line implantation Group 1 – Irinotecan 100 to 500 mg/kg Group 2 – Irinotecan 50 to 1,000 mg/kg Group 3 - Control	 IV IP IV, IP	Control animals died after 13.3 ± 4.2 days. Early toxic deaths occurred after IV injection of 400 mg/kg and 600mg/kg and IP administration of 800 mg/kg and 1000mg/kg
(42)	BALB/c *nu*$^{+}$/*nu*$^{+}$	Not reported	8 weeks	5FU – 10, 40, 80mg/kg Mitomycin C – 0.08, 0.32, 0.64mg/kg Adriamycin – 0.98, 3.2,	IV IV IV	Only 33% of the 5-FU with cisplatin and epirubicin group died (n=8-12). All other mice survived.

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				6.4mg/kg Epirubicin - 1.2, 4.8, 9.6mg/kg Carboquone - 0.16, 0.64, 1.28mg/kg Cisplatin – 0.9, 3.6, 7.2mg/kg Carboplatin – 9.0, 36.0, 72.0mg/kg Etoposide – 3.0 ,12.0, 24.0mg/kg (See Figure 2)	 IV IV IV IV IV	No significant body weight ratio changes for the single agent experiment. 5-FU with adriamycin, 5-FU and epirubicin, 5-FU and etoposide, 5-FU with cisplatin and epirubicin and 5-FU with mitomycin C and epirubicin all showed significant body weight ratio reduction compared with the control. All other combinations did not show any significant change.
(43)	BALB/c	Female	2-month	Group 1 - 5FU alone - 100 mg/kg^{+} once weekly in combination with Leucovorin - 50 mg/kg once weekly given at 1 h before and simultaneously with 5FU^{+}. Group 2 - Cisplatin - 9 mg/kg once weekly	IP IP	100 mg/kg 5FU alone caused 80% deaths (data not shown) and 5FU alone was thus given at a safe dose of 80 mg/kg
(44)	BALB/c	Female	6-7 weeks	Group 1 - 5FU 80mg/kg weekly for 3 weeks^{+} Group 2 - 5-fluoro-2-deoxyuridine 200mg/kg weekly for 3 weeks^{+} Group 3 - 5-fluoro-2-deoxyuridine 400mg/kg weekly for 3 weeks^{+} Group 4 - Control	IP IP IP	Not reported
(45)	BALB/c	Male	8 weeks	Group 1 – Control		Not reported

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				Group 2 - Doxorubicin 10mg/kg day once weekly for 2 weeks[+] Group 3 – Control Group 4 - 5FU 20mg/kg daily for one week[+]	IV IV	
(46)	BALB/c *nu/nu*	Female	8-12 weeks	Group 1 - Doxorubicin 6 mg/kg weekly for 3-4 weeks Group 2 - Doxorubicin 10 mg/kg weekly for 3-4 weeks	IV IV	Not reported
(47)	Cba/CaJ	Male	Not reported	5FU (no dose specified)	IP	Not reported
(48)	BALB/c *nu/nu*	Not reported	Not reported	Group 1 - 5FU 90mg/kg, BCNU 24mg/kg, Doxorubicin derivatives (0-DX) 6.5mg/kg and DeoDX 6mg/kg weekly for 3 weeks [+] Group 2 - 5FU 100mg/kg, BCNU 24mg/kg, 0-DX 4.5mg/kg and DeoDX 4mg/kg weekly for 3 weeks [+]	IV IV	"Mice did not loose more than 10% total body weight"
(49)	BALB/c nude	Female	5 weeks	Group 1 - 5FU 10mg/kg weekly for 3 week and Vincristine 0.05mg/kg once only	IP	5 of 15 died – no other details
(50)	CBA	Male	6 weeks	Group 1 – Control Group 2 – Cyclophosphamide 180mg/kg	IP IP	Not reported
(51)	CBA/lac	Male	6 weeks	Cyclophosphamide 50-200mg/kg, 5FU 50-200mg/kg, CCNU 17.5-35mg/kg, Actinomycin D 0.075-0.3mg/kg, cis-DDP 3-6mg/kg, Pentamethylmelamine 50-	IP	Not reported

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				100mg/kg, Doxorubicin 10-15mg/kg (unable to distinguish groups)		
(52)	CBA/lac	Male and female	6 weeks	5FU 30mg/kg daily for 5 days, MeCCNU 20-25mg/kg single dose, Melphalan 8mg/kg single dose, Actinomycin D 0.25-035mg/kg single dose, Hexamethylmelamine 5mg/kg daily for 5 days, Methotrexate 5mg/kg daily for 5 days, Cyclophosphamide 200mg/kg single dose, Cis-dichlorodiamino-platinum 3mg/kg daily for 5 days* (8 types of chemotherapy per cell line – no groups identified)	IP	None reported
(53)	BALB/c C57B1/6	Female	Not reported	Various (See Appendix –S2)	IP, IV	Toxicity deaths reported (See Appendix – S2)

[+] Chosen cancer therapy was either reported as investigated separately with a dose escalating regime up to the maximum tolerated dose or referenced

* Chosen cancer therapy was tested in preliminary experiments but these data were not shown

MTD – maximum tolerated dose

5FU – 5-Fluorouracil

IV – intravenous administration, IP – intraperitoneal administration, PO – oral administration

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Table 3 - A summary of all the studies included describing primary outcomes, key findings with the effects observed on the CRC model and the techniques/ methodology utilised to interrogate the effects observed

Reference Number	Primary outcome	Effects observed	Methodology
(9)	To evaluate the efficacy of regorafenib in combination with cetuximab to overcoming resistance to anti-EGFR monoclonal antibodies by using different human colorectal cancer cell models.	Mice receiving cetuximab or regorafenib alone had large tumors with 80% and 70% incidence of lymph node metastases respectively. The combined treatment strongly inhibited the tumor growth. Mice treated with vehicle, cetuximab or regorafenib were large and highly vascularized, whereas cetuximab plus regorafenib-treated mice developed small tumors without evidence of neovascularization.	Tumour volume, immunohistochemistry
(10)	To study serum phospho-CSE1L for assaying the efficacy of targeted therapy using colorectal tumor xenograft models and drugs including sorafenib, and lapatinib.	Serum phospho-CSE1L can be used to monitor the efficacy of targeted drugs as early as 3 days after drug administration. The results suggested that serum phospho-CSE1L has clinical application in early detecting the development of resistance to targeted drugs to improve the cure rate of cancer. Sorfenib and lapatinib were effective at inhibiting tumour growth.	Tumour volume and phosphor-CSE1L ELISA
(11)	To study the antitumor effects of a combination of local injection with anti-CD137 mAb and intermittent low-dose chemotherapy using cyclophosphamide and gemcitabine in CT26 colon carcinoma.	Combination therapy suppressed the growth of mAb untreated tumours. The results suggest that intermittent immunochemotherapy using cyclophosphamide and gemcitabine could retain the therapeutic potential of anti-CD137 mAb that is normally impaired during the late tumour bearing stages.	Tumour volume, flow cytometry to assess the expression of CD137 on CD4 and CD8 T cells

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(12)	Confirm the feasibility of alternate- day administration of 5-FU/Leucovorin in an vivo xenograft tumor models	The tumour growth inhibition in the alternate-day group was better than in the daily group, possibly resulting from apoptosis due to the suppression of cIAP2 but not XIAP. Body weight loss was observed only in the daily group, with the most severe loss observed on day 15; however, from days 15–28, which was the drug-free interval, the body weight in the daily group started to increase gradually until there was no significant differences in the BWC between the three groups (day 28).	BWC %, tumour volume, tumour growth inhibition (TGI) Mucosal injury and myelosuppression compared the lengths of the villi and the leukocyte counts among these groups. Immunohistochemistry of HT-29 tumors after 5-FU/Leucovorin administration
(13)	To gain an understanding of 5FU and irinotecan adverse effects on host immunity.	Comparing the effects of mono- or combination 5FU and irinotecan revealed that the irinotecan component was harmful, yielding a strong immunosuppression mediation via myeloid-derived suppressor cells, with rapid disease progression and decreased survival as compared with the beneficial effects of 5FU alone	Overall survival and tumour volume
(14)	To determine whether rapamycin treatment could	Rapamycin might inhibit the mTOR/Bcl2 pathway and increase chemosensitization of tumor cells.	TUNEL assay, PCNA and Bcl2 staining, western blotting and tumour volume

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	inhibit the mTOR pathway and whether rapamycin combined with 5-FU would has a synergistic effect	Rapamycin alone only slightly inhibited tumor proliferation and mitosis without a significant difference. Rapamycin slowed tumor growth in the early-treatment experiment with rapamycin by about 48% compared with the control group. When rapamycin was combined with 5-FU, the inhibition rate increased to 60%. Rapamycin combined with 5-FU significantly suppressed Bcl2 expression, and dephosphorylated S6K, synergistically suppressing tumor proliferation and mitosis.	
(15)	Dovitinib may attempt to boost therapeutic kill by employing combination regimen with oxaliplatin.	The combination of two drugs showed a significant decrease in tumor growth stating from an early stage as compared to vehicle or oxaliplatin treatment while at late stage compared to dovitinib alone. Inhibition of tumor growth in HT-29 tumor model with coordinating decrease in the expression of Ki-67 (biomarker for proliferation) and CD31 (biomarker for angiogenesis). The decrease was more pronounced in the combination group as compared to either of the groups alone.	Tumor volumes, immunohistochemical expression of Ki-67 and CD-31.
(16)	To interrogate the interplay between chemotherapy and cancer initiating cells	Chemotherapy induces remodelling of the tumor microenvironment to support the tumor cellular hierarchy through secreted factors	Tumour volume, immunofluorescent imaging
(17)	To interrogate the therapeutic synergy of ipilmumab when combined	Strong therapeutic synergy was observed when ipilmumab was combined with standard chemotherapeutic options. Ipilmumab and	Percentage tumour growth inhibition, days for cancer to reach target size, percentage complete response

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	with ixabepilone, paclitaxel, etoposide and gemcitabine	etoposide generated a memory immune response that leads to tumour rejection in mice rechallenged with tumour cells.	
(18)	To study the utility of several functioning imaging modalities to assess the efficacy of bevacizumab	Continuation of cevacizumab inhibited tumour growth even after disease progression. Bevacizumab after progression resulted in significant changes in tumour proliferation and microenvironment.	Tumour volume, DCE-MRI and FDG-PET, immunohistochemistry
(19)	To study the activity of regorafenib in comparison with the angiogenesis inhibitor DC101 in the highly aggressive murine CT26 CRC model	Regorafenib exerted the strongest antitumor effect when compared to DC101 especially after day 7 tumour post-implantation.	Tumour volumes measured by MRI
(20)	To examine the effect of combining cediranib with mechanistically distinct anti-tumour therapies	The combination of cediranib and other anti-tumour agents inhibited tumours to a greater extent that corresponding monotherapies. The combination of cediranib with gemcitabine or irinotecan inhibited tumour growth profoundly	Tumour volume
(21)	To investigate the use of liposomal irinotecan +/- 5FU	Treatment with irinotecan (60mg/kg) delayed the time required for a 5-fold increase in tumour volume. Liposomal irinotecan and 5FU combined delayed this further. A comparable result was achieved with liposomal irinotecan alone (40mg/kg)	Tumour volume
(22)	The impact of sorafenib single treatment versus combination treatment in	Expression rates of receptor tyrosine kinases VEGFR1 and PDGFRβ as well as of the ligand PDGFA were decreased by all treatment regimens	Immunohistochemistry - Ki-67, PDGFA, VEGFA, VEGFR1, VEGFR2, PDFGRα, and PDGFRβ protein expression, Tumour

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	human colorectal cancer.	used. However, no significant differences were detected between treatment groups. Sorafenib monotherapy was at least equally effective as the 5-FU monotherapy or as the combination therapy and even tended to inhibit in vivo tumor growth somewhat better than the combination therapy	size
(23)	To investigate responses to EGFR and VEGF targeted therapies	Heterogenicity in colorectal cancer may result in differences in responses to dual-inhibition of EGFR and VEGF	Tumour size and relative tumour growth inhibition
(24)	Optimal combination schedule and antitumor activity of oral S-1 with oxaliplatin combination therapy (SOX) against human colorectal cancer xenografts in vivo.	The divided administration of oxaliplatin was optimal for increasing the antitumor activity, while obtaining a lower toxicity compared with other schedules. SOX may be useful against colorectal cancer in a manner equivalent to that of FOLFOX or COX but with a greater convenience and at a lower cost. The intermittent administration of oxaliplatin may further accelerate the effects of SOX.	TGI, BWC; %, growth delay period (GDP), which indicates the difference in the period during which the relative tumor volume grew to 4 (corresponding to 50% of the size of the control tumors at the endpoint on day 22) and toxicity, defined as a 20% or more body weight loss or toxic death.
(25)	Toxicological, pharmacokinetic, biodistribution, and efficacy evaluations of Fluorodex (FD) compared with 5-FU: Leucovorin was undertaken. These were compared with the dose-matched sequential administration of 5-FU: Leucovorin.	Fluorodex (FD) showed bioequivalence to 5-FU:Leucovorin as assessed by the tissue distribution and pharmacokinetic studies of 5-FU, but was generally better tolerated as determined by weight loss, hematological, and other clinical parameters. Furthermore, using human carcinoma tumor models in mice, FD resulted in equivalent or improved efficacy profiles compared with 5-FU: Leucovorin acid. These all-in-one formulations represent a superior injectable form of 5-FU that allows co-delivery of Leucovorin. This should translate into improved patient tolerability with potential for enhanced efficacy.	Dose-limiting toxicity endpoints, histopathological analysis of hematoxylin and eosin-stained and oil red-O-stained sections of livers, kidneys, and spleens (for some experiments), tumour growth delay, pharmacokinetic and biodistribution analyses, tumour volume

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(26)	To investigate the effect of leucovorin on the antitumor activity of UFT and/or 5-FU prodrugs in low folate diet-fed nude mice using human colorectal cancer xenografts with various expression levels of thymidylate synthase (TS).	The addition of leucovorin (LV) to UFT resulted in a 55-79% inhibition of tumor growth among 11 types of colorectal tumor xenograft, whereas UFT alone showed 23-67% antitumor activity. Although there was an inverse relationship between the antitumor effect of UFT alone and UFT plus LV and tumoral TS activity, UFT plus LV appeared to have a more potent antitumor effect than UFT alone on colorectal tumours such as Co-3 and KM12C/5-FU with high expression levels of TS. This finding was confirmed by the significant positive correlation between the relative inhibition ratio of UFT/LV to UFT alone and TS levels in tumours. Results indicate that a combined therapy of UFT with LV may contribute to the treatment of colorectal cancer patients with low and high expression levels of tumoral TS by increased formation of the ternary complex of TS leading to potentiated antitumor efficacy of UFT.	Tumour volume, relative tumour volume, inhibition ratio, TS binding assay, thymidine phosphorylase assay, folate assay.
(27)	To study the synergistic effect of 3-MA and 5-FU in vivo	Treatment with 3-MA alone had no significant influence on tumour growth. On day 35, mean tumour volume and mean tumour weight of combination group mice were significantly reduced by 66.8% and 49.3% compared with the 5-FU group. Combination therapy increased activated caspase-3 12.4% compared with treatment of 5-FU alone. These results demonstrate that use of the 5-FU, 3-MA combination also improves the effect of 5-FU on colon cancer in vivo.	Tumour volume, tumour weight, tumour tissue protein extraction and subjected to immunoblotting (of caspase-3, LC3, and p62).
(28)	A conditional *Msh2* disruption (*Msh2*^{LoxP}),	*VCMsh2*$^{LoxP/null}$ tumors were predominantly resistant to both chemotherapies, and only a small	The number of tumors and their location was recorded under a dissecting

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	permitting tissue-specific gene inactivation was generated and interrogated. We combined the *VCMsh2^{LoxP}* allele with either *Msh2*Δ*7null* (*VCMsh2$^{LoxP/null}$*) or *Msh2^{G674D}* mutations (*VCMsh2$^{LoxP/G674D}$*) to create allelic phase mutants.	number of tumors showed growth retardation. In contrast almost all tumors in *VCMsh2$^{LoxP/G674D}$* mice who responded well to either cisplatin or FOLFOX treatment regimen MRI based *in vivo* measurements of tumor growth showed that the intestinal tumors in *VCMsh2$^{LoxP/G674D}$* animals were sensitive to both cisplatin and FOLFOX, while tumors from *VCMsh2$^{LoxP/null}$* mice were generally resistant to both drugs.	microscope. Histological analysis, tumors were stained with hematoxylin and eosin. Relative tumor size was measured using a Vernier Caliper with fine adjustment. Tumors were measured by magnetic resonance imaging.
(29)	Exploring the therapeutic potential of combined in vivo treatment of CRC models with a 5FU oral prodrug and leucovorin vs. conventional 5FU and leucovorin	The oral prodrug/leucovorin combination increases anticancer activity compared to prodrug alone or 5FU/leucovorin combination	Tumour volume, tumour growth inhibition and body weight change
(30)	To determine the antitumor activity and tolerability of capecitabine at its maximal tolerated dose (MTD) and ⅔ MTD administration in a traditional 14/7 schedule or a 7/7 schedule alone and in doublet or triplet combinations.	In mice bearing moderately thymidine phosphorylase–expressing HT29 or Colo205 colorectal xenografts, a capecitabine 7/7 schedule permits increased drug delivery compared with traditional 14/7 regimens, greatly improving monotherapy activity without major toxicity. Adding bevacizumab to capecitabine 7/7 resulted in significantly greater survival relative to either agent alone. Addition of oxaliplatin increased efficacy and significantly better with triplet combination.	Tumour volume, tumour growth inhibition, cumulative survival and increased life span

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(31)	To understand the effects of combination treatments in animal models showing antitumor activity of bevacizumab as a monotherapy and in combination with capecitabine or capecitabine and oxaliplatin	Bevacizumab showed significant antitumor activity as a monotherapy in all three models. The microvessel density (MVD) in tumor tissues treated with bevacizumab was lower than that of the control. Antitumor activity of bevacizumab in combination with capecitabine was significantly higher than that of each agent alone (COL- 16-JCK, COLO 205). The antitumor activity of bevacizumab in combination with capecitabine + oxaliplatin was significantly superior to that of capecitabine + oxaliplatin (COL-16-JCK). Thymidine phosphorylase and VEGF levels were not increased by bevacizumab or capecitabine, respectively, suggesting there are other potentially efficacious mechanisms involved.	Immunohistochemistry - thymidine phosphorylase and VEGF levels MVD was determined as the ratio of the CD34-positive area to the total observation area. Four to six fields per section (0.4977 mm^2 each) were randomly analyzed, excluding necrotic areas.
(32)	To assess if antitumor activity of capecitabine can be increased by modifying its dosing schedule	Modifying the capecitabine schedule from 14/7 to 7/7 improved the antitumor efficacy of doublet and triplet combination regimens. The survival benefits of 7/7 *versus* 14/7 schedules and the addition of bevacizumab to capecitabine and irinotecan were biologically significant	Tumor growth inhibition (TGI), survival and mean increase in lifespan (ILS), average percentage weight change.
(33)	To establish whether cetuximab, has the potential to restore responsiveness to oxaliplatin in preclinical cancer models	Oxaliplatin + cetuximab efficacy was greater than that of monotherapies and independent of the responsiveness to oxaliplatin monotherapy.	Tumors volumes, treated versus control percentage (T/C%)
(34)	To evaluate the antitumor	Anti-tumour activity of the combination at two-thirds	ELISA (dThdPase activity),

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	activity of capecitabine and oxaliplatin in colorectal cancer xenograft models	of the maximum tolerated dose was superior to that of each monotherapy at the maximum tolerated dose.	immunohistochemistry and tumour volume
(35)	To investigate the efficacy and safety of cetuximab combined with standard oxaliplatin-based chemotherapy in the first-line treatment of epidermal growth factor receptor–expressing colorectal cancer.	The supra-additive effects of cetuximab and oxaliplatin were confirmed in this xenograft model and reconfirmed in a phase II patient study.	Tumour volume
(36)	To examine the antitumor activity and tolerability of erlotinib and capecitabine in human colorectal, cancer xenograft models.	Erlotinib inhibits tumor growth in colorectal cancer human tumor xenograft models Erlotinib and capecitabine demonstrated at least additive activity in the LoVo tumor model. The antitumor activity of the combination was greater than that of capecitabine alone at the MTD Erlotinib treatment did affect the thymidine phosphorylase (TP) as confirmed immunohistochemically	Thymidine phosphorylase (TP) and dihydropyrimidine dehydrogenase (DPD) levels in tumor tissues HER1/EGFR protein levels in tumor tissues Tumor volume, body weight, tumor-growth inhibition
(37)	Studies of the antitumor activity and toxicity of capecitabine or irinotecan alone and in combination with each other in human tumor xenografts of colorectal carcinoma using clinically relevant	HT-29 xenografts were de novo resistant to capecitabine and irinotecan alone at the MTD, whereas HCT-8 xenografts were relatively more sensitive, yielding 10%-20% cures. The combination of irinotecan/capecitabine was much more active than either drug alone against both tumor models. The cure rates were increased from 0 to 20% in HT-29 xenografts and from 10%-20% in HCT-8 tumor	Tumour volume, tumour weight

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	schedules.	xenografts, respectively. Irinotecan/capecitabine had clear advantage over irinotecan/5-FU in efficacy and selectivity in that they were more active and less toxic. The extent of synergy with irinotecan/capecitabine appears to be tumor-dependent and independent of the status of p53 expression.	
(38)	5FU drug uptake and metabolism	Both tumour variants generated MRS-detectable 5-FU nucleotides and showed similar initial growth inhibition after treatment. However, the growth rate of C26-B tumours returned to normal, while the sensitive C26-10 tumours, which produced larger fluoronucleotide pools, still showed moderate growth inhibition. Carbogen breathing did not significantly influence 5-FU uptake or fluoronucleotide production but did significantly enhance growth inhibition in C26-10 tumours. While both tumour variants exhibited incorporation of 5-FU into RNA and inhibition of thymidylate synthase (TS), clearance of 5-FU from RNA and recovery of TS activity were greater for the insensitive C26-B line, indicating that these processes, in addition to 5-FU uptake and metabolism, may be important determinants of drug sensitivity and treatment response	Time courses for tumour growth, thymidylate synthase activity, 5-FU in RNA, ^{19}F MR Spectroscopy-detected 5-FU metabolism and tumour growth curves
(39)	The effect of oxalilpatin and topotecan in combination on a sequential schedule from cell culture to clinical setting	Treatment with topotecan alone caused 60% inhibition of growth at the end of treatment compared with the other groups. Two agents in combination markedly inhibited tumor growth – 7 weeks following tumor injection, tumor volume was 90% smaller than that observed in mice treated with topotecan alone and tumors did not	Tumour size

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		achieve a size incompatible with normal life until at least 5 weeks after treatment withdrawal.	
(40)	To asses the inhibition of tumor growth by oxaliplatin combined with UFT and folinic acid (FA).	Combined treatment (UFT+FA+oxaliplatin) reduced tumor weight by 39% compared to oxaliplatin alone or UFT+FA Pathological examination of the tumors showed no difference between the four groups.	Toxicity was evaluated in terms of mortality and the body weight ratio. Tumor size (i.e. surface area) and body weight. Successive tumor measurements were normalized in relation to the initial (baseline) measurement per animal in order to establish growth curves for each group.
(41)	A comparison was made of the efficacy and pharmacokinetics of irinotecan after IP and IV administration to mice.	The toxic deaths related to irinotecan administration occurred immediately or soon after injection and the symptoms suggested a cholinergic syndrome.	Antitumor activity was determined by the median survival time. Toxic deaths and immediate toxic deaths were defined as deaths occurring at less than 7 days after the end of the treatment and less than 24h after an injection, respectively.
(42)	To evaluate the efficiency of various combination chemotherapies, including 5-FU, on human colorectal cancer xenograft lines (CC-KK and RC-TK)	When administered singly, 5-FU, Epirubicin, Carboquone were effective against CC-KK. 5-FU, Mitomycin C, Epirubicin, Adriamycin, Carboquone and Carboplatin were effective against RC-TK. Of the two agent combinations, 5-FU, Cipslatin and 5-FU was the most effective against both CC-KK and RC-TK. Of the three agent combinations, only Cisplatin with 5-FU and Mitomycin C was the most effective against both cell lines, more so than Cisplatin+5-FU; suggesting it is the most useful regime.	Inhibition ratio, loss of body weight, mortality, relative tumour growth ratio, % inhibition of the tumor growth ratio.
(43)	To assess the relationship between tumor size (cutoff	The antitumor effect of 5-FU in these large tumors was decreased and could not be modulated by	The antitumor activity was evaluated by analysing

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	point 200 mm^3) and the antitumor activity of 5-FU and its modulation by leucovorin in murine Colon 26 and Colon 38 tumors.	leucovorin. In addition, three subtypes of Colon 26 (Colon 26-A, Colon 26-B, and Colon 26-10) were identified and characterized for tumor-induced weight loss, thymidylate synthase (TS) activity, response to chemotherapy, and histological features. Among untreated tumors, TS catalytic activity was highest in Colon 26-B. The antitumor activity of 5-FU could be modulated by leucovorin in Colon 38, Colon 26-10, and Colon 26-A but could not in Colon 26-B, with complete responses (CR) being obtained in Colon 26-10 and Colon 38. The latter two were highly sensitive to cisplatin. Further-more, necrosis was noted in Colon 26-B and Colon 38 but not in Colon 26-A.	(a) The tumor- doubling time (TD), (b) The growth-delay factor (c) The maximal mean tumor volume (d) The percentage of complete regressions (CR) (e) Tumour volume
(44)	To delineate the biochemical mechanism associated with the observed differences in antitumour activity of 5FU and 5-fluoro-2-deoxyuridine	The antitumor activity of FdUrd was superior to 5FU not only at the MTD but also at equimolar doses, 200 mg/kg FdUrd versus 80 mg/kg 5FU. Administration of 200 mg/kg FdUrd resulted in a tumor-doubling time of 19 days and 13% complete tumor regressions; after 80 mg/kg 5FU, these values were 7 days and 0%, respectively.	Tumour volumes, 5FU and FdUrd concentration measurements by gas chromatography, tumour TS inhibition, determination of RNA incorporation by gas chromatography and mass spectrometry
(45)	To determine whether the organ micro-environment could influence the response of a murine CT-26 colon carcinoma to systemic therapy with doxorubicin and 5FU	We found differences in sensitivity of CT-26 tumors growing in the subcutis, spleen, liver and lung to doxorubicin and 5-FU. The sensitivity of the CT-26 cells to doxorubicin was highest in the subcutaneous environment, intermediate in the spleen and the cecum, and lowest at metastatic sites such as the liver and lungs. Different patterns of organ-specific chemosensitivity were found for 5-FU. CT-26 cells growing in the lung were most sensitive to systemic administration of 5-FU; those growing in the	Growth inhibition %, tumour volume, Cyto fluorescence and radiolabelled 5-FU extraction

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		subcutis, the spleen and the cecum demonstrated intermediate sensitivity; those in the liver were resistant. Organ-site-associated differences in drug sensitivity to either doxorubicin or 5-FU were not associated with drug distribution patterns in the tumors.	
(46)	To test the anti-tumour activity of doxorubicin against different types of human tumours	No anti-tumour effect was demonstrated on colon cancer	Tumour volume
(47)	To examine the relationships between free 5FU, thymidylate synthetase and the ability for ternary complex formation.	High levels of TMP synthetase activity may be responsible for innate insensitivity of some xenograft cell lines, with substantial variability between tumours.	TMP synthetase activity, tumour volume, free FdUMP levels
(48)	To investigate the activity of clinically useful chemotherapeutic agents against colorectal tumours. In addition chemosensitivity to 5FU is compared to Doxorubicin and new doxorubicin derivatives.	77% (5/9) of the colorectal tumors were biologically sensitive to the treatment with 5-FU but the percentage of statistically significant sensitive tumors was 22%. BCNU was have found to be sensitive in 33 %. Results suggest that the two new doxorubicin derivatives, 4'-deoxydoxorubicin and 4'-O-methyl- doxorubicin, should be more active in the patient than both of the clinically used drugs, 5-FU and BCNU.	Tumour volume
(49)	To determine the sensitivity of 5FU and vincristine on human adenocarcinoma	5 of 6 tumours showed a significant reduction in size following combined therapy	Tumour volume

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	tumours xenotransplanted into mice.		
(50)	Investigation of inter-tumour variation in the xenograft system might affect the design of chemotherapy experiments	Marked inter-tumour variation in growth rates was observed. Whereas growth delays of one doubling time can be detected with only 6 mice per group, far more mice (23) are required to detect difference of half a doubling time. With 2 tumours per animal a considerable saving in numbers of mice is achieved.	Tumour volume
(51)	Investigation of colorectal tumour responses/ chemosensitivity to cytotoxic agents	The chemosensitivity of each tumour line to a spectrum of agents was individual, and no pattern of response which would allow prediction of individual agent efficacy was apparent. Cyclophosphamide, methyl-CCNU and 5-fluorouracil produced marked growth inhibition in individual tumour lines, whereas actinomycin-D, cis-dichlorodiammine platinum, doxorubicin and pentamethylmelamine showed little activity.	^{3}H-thymidine fractional incorporation
(52)	To measure the range of responses among 10 difference colorectal tumours	Average response corresponded to a delay of only about 0.5 doubling times with a heterogeneous chemotherapeutic response.	Tumour volume, growth delay
(53)	Evaluation of new and potentially useful chemotherapeutic agents and combinations of agents against advanced-stage tumors	The most impressive anti-tumor activity was obtained with anguidine and the combination of anguidine + 5-FU against colon adenocarcinoma. Antitumor potentiation was obtained when the combination was injected simultaneously on a Q7d schedule, but not on an alternating schedule of administration (3 or 4 days separating injections of the two agents). Anguidine was highly active only in	Tumour free survival, tumour regression, tumour volume, tumour weight, tumour growth delay, tumour growth inhibition

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		a colon tumor that was very responsive to 5-FU. The correlation between high 5-FU sensitivity and high anguidine sensitivity may be coincidental.	

BWC % - Body weight change was calculated using: BWC (%) = [(body weight on a given day/mean body weight on day 0) – 1] x 100

TGI - tumor growth inhibition (TGI) was calculated using: TGI (%) = [1- mean tumor volume of the treatment group/mean tumor volume of the control group] x 10

MTD – maximum tolerated dose
5FU – 5-Fluorouracil
IV – intravenous administration, IP – intraperitoneal administration, PO – oral administration

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Table 4 - Proposed minimum core outcome dataset for the reporting of methodology and results from pre-clinical in vivo animal experimentation.

Core outcome Heading	**Core outcomes reported**	**Section reported**
Animal characteristics	Number of animals used per group	Methods
	Animal strain	Methods
	Gender	Methods
	Age (in weeks) at initial treatment	Methods
Cancer and chemotherapy characteristics	Animal model description (xenograft, orthotopic etc.)	Methods
	Cancer cell line	Methods
	Origin of cancer cell line	Methods
	Number of cancer cells injected	Methods
	Name of chemotherapy used	Methods
	Dose (mg/kg or mg/BSA)	Methods
	Number of cycles	Methods
	Route of chemotherapy administration	Methods
	Overall length of study from initial chemotherapy treatment	Methods
	Origins of the selected chemotherapeutic regime	Methods
Adverse events	Definition of acceptable weight loss before culling or rescue	Methods
	Definition of adverse event	Methods
	Number of events reported	Results
	Severity	Results

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	Unexpected deaths	Results
	Any extraordinary measures of support if adverse event encountered	Results