Improving the reporting of death and investigating the role of probiotics in mucositis and infections in children with cancer

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Intellectual Property and publication Statements

The candidate confirms that the work submitted is her own, except where work which has formed part of jointly authored publications has been included. The contribution of the candidate and the other authors to this work has been explicitly indicated below. The candidate confirms that appropriate credit has been given within the thesis where reference has been made to the work of others.

The following jointly authored publications have been written as a result of the work in this thesis:

1. Hassan, H., Rompola, M., Glaser, A.W, Kinsey S, Phillips R. The efficacy and safety of probiotics in people with cancer: a systematic review and meta-analysis. Supportive Care Cancer (2018) 26: 2503.

Contribution: H Hassan was responsible for the creation of the question, review design, literature search, data extraction, data analysis and writing the first and subsequent drafts.

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Abstract

This thesis examines the classification of death, and the use of probiotics to reduce and prevent infection-related mortality in children diagnosed with cancers. It comprises of three main parts.

The first part describes a study that validated a consensus-based definition of treatment-related mortality (TRM) and cause-of-death attribution system. This took place in a single institution in Leeds, the UK outside the centre it was initially developed (Toronto, Canada). Two consultants and two clinical research associates independently classified deaths as TRM or “not treatment-related” according to an algorithm. When TRM occurred, reviewers applied the cause-of-death attribution system, and inter-relater reliability was then assessed. This study demonstrated that the classification and cause of death attribution systems can be implemented in different health care settings, but that further research is required for patients receiving palliative care.

The second part of this thesis describes a systematic review and meta-analysis that investigated the efficacy and safety of probiotics in people with cancer. Probiotics appear safe to deliver and may reduce the incidence of diarrhoea and duration of fever, but, heterogeneity, unclear bias, and a lack of paediatric participants demonstrated uncertainty in these findings. Findings from this systematic review were used to develop a randomised-controlled feasibility study.

The third part reports the first study undertaken to evaluate the feasibility of undertaking an randomised-controlled trial (RCT) to investigate the use of probiotics (Symprove) to prevent or reduce mucositis and infection in children with cancers in the UK. Evaluation suggested that a RCT is feasible, but further considerations are needed to address significant barriers to recruitment and adherence to the capture of data that were identified. Findings from this study have been used to develop a parallel biological sub-study that can be undertaken in a future RCT.

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Abbreviations

5-FU	5-fluorouracil
AAD	Antibiotic-associated diarrhoea
AE	Adverse event
ALL	Acute lymphoblastic leukaemia
AML	Acute myeloid leukaemia
ATRT	Atypical teratoid rhabdoid tumour
BSBL	Broad-spectrum beta-lactam
ChIMES	Children's International Mucositis Evaluation Scale
CI	Confidence interval
CML	Chronic myeloid leukaemia
CNS:	Central nervous system
COMET	Core Outcome Measures in Effectiveness Trials
CONSORT	Consolidated Standards of Reporting trials
CRA	Clinical research assistant
CRD	Centre for Reviews and Dissemination
CRP	C-reactive protein
CT	Computed tomography
CTIMP	Clinical Trials of an Investigational Medicinal Product
DIPG	Diffuse intrinsic pontine glioma
EBV	Epstein-Barr Virus
ECG	Electrocardiography
ESMO	European Society for Medical Oncology
FAO	Food and Agriculture organization
HCP	Health Care Professional
HD	High dose
HR NBL	High-risk Neuroblastoma

HRA Health Research Authority

HSCT Haematopoetic stem cell transplant

ICBN The International Code of Botanical Nomenclature

ICD International Statistical Classification of Diseases and Related Health Problems

IL Interleukins

IMP Investigational medicinal product

IQR Interquartile range

IRM Infection-related mortality

I-ROBINS Risk of Bias in Non-randomised Studies of Interventions

IV Intravenous

K Kappa statistic

LLN Lower limit of normal

LTHT Leeds Teaching Hospital Trust

MASCC Multinational association of supportive care in cancer

MDT Multi-disciplinary team

MHRA Medicines and Healthcare products Regulatory Agency

MRI Magnetic Resonance Imagining

NBL Neuroblastoma

NCI CTCAE National Cancer Institute Common Terminology Criteria for Adverse Events

NCT Non-curative therapy

NEC Necrotising Enterocolitis

Neut Neutrophil

NG Nasogastric

NHS National Health Service

NICE National Institute for Health and Care Excellence

NIHR National Institute of Health Research

OMWQ-HN	Oral Mucositis Weekly Questionnaire - Head and Neck cancers
OR	Odds ratio
PCAS	Patient-controlled analgesia
PI	Prediction interval
PIL	Participant information leaflet
PPI	Patient and public involvement
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROM	Patient-Reported Oral Mucositis Symptom
R&D	Research and development
RCT	Randomised controlled trial
REC	Research ethics commit
RR	Risk Ratio
SAE	Serious adverse vent
SEER	Surveillance, Epidemiology and End Results
SSPedi	Self-report Symptom Screening in Paediatrics tool
SWAT	Studies Within a Trial
TKI	Tyrosine enzyme inhibitor
TPN	Total parental nutrition
TRM	Treatment-related mortality
ULN	Upper limit of normal
ULN:	Upper limit of normal
UTI	Urinary tract infection
VOD	Vaso-occlusive disease
WCC	White cell count
WHO	World Health Organisation.

1 Introduction

1.1 Background

Childhood cancers are relatively rare accounting for less than 1% of cancer cases diagnosed in the UK between 2011-2013 (1) and survival of children diagnosed with cancers have increased during recent decades (2). Before the 1950s, most children diagnosed with cancers did not survive. Sixty years later, 5-year survival is reported to be approximately 80% in developed countries (3).

This success is mostly credited to the development of treatment protocols which have progressed to include multi-modal and combination therapies, treatment stratification according to prognostic factors, and improved supportive care strategies. Recently, personalised interventions developed to target tumour biology have also been shown to improve survival in malignant conditions further. For example, imatinib a tyrosine kinase enzyme inhibitor (TKI) is now used to target the PCR-ABL translocation mutation between chromosomes 9 and 22 in Philadelphia positive chronic myeloid leukaemia (CML) and acute lymphoblastic leukaemia (ALL). Prior to the use of TKIs, patient with these specific mutations received combination chemotherapy regimens which achieved a complete response rate of 45-90% with few long term survivors (4). However, a trial in children with good-risk Philadelphia ALL reported that 4-year overall survival was 75 % (95% confidence interval (CI) 61%–84·9%) for those receiving imatinib and 56% (95% CI 36%–72%) for those who did not receive imatinib (p=0·06) (5). Because of these findings, children with Philadelphia positive ALL now have the option of receiving imatinib as part of first-line treatment.

However, despite the improvement of survival rates, it is estimated that 20 % of children diagnosed with cancers die (6). Between 2015 and 2017, 236 children died from cancers in the UK alone (7, 8) and it is reported as the second most cause of death of children in developed countries (9). An estimated 80,000 children worldwide are believed to die from cancer every year. This has been attributed to the following reasons:

- Lack of improvement in anti-cancer treatment efficacy. Several tumour types are disproportionately unaffected by the development of modern medicine. For example, diffuse intrinsic pontine gliomas (DIPGs) still have dismal outcomes: 1-year overall survival of less than 40% (10). Sadly, this has not improved during recent decades.
- Toxicity from treatments utilised. Despite improved supportive care strategies, toxicity from treatment is still a significant cause of mortality.

Identifying the cause of death in children with cancer is vital in enabling the analysis of existing data while directing future research appropriately for different cancers. Unfortunately, there are no universally validated methods to distinguish deaths caused directly by disease from deaths which occur due to toxicities. For cancers with poor survival, outcomes may require research to focus on curative strategies whilst cancers with good survival outcomes, but have a significant proportion of deaths from toxicity of interventions may require greater focus on supportive care interventions.

Death not directly due to cancer has been termed “treatment-related mortality” (TRM) and causes include infection, bleeding, and organ dysfunction (11). Infection is recognised as a leading cause of morbidity in TRM and poses a significant risk in patients diagnosed with malignant haematological conditions (16).

Therefore, the research undertaken for this thesis will be addressed in two parts:

- The first part will focus on how the reporting of cause of death in children diagnosed with cancer can be improved to enhance current interventions and direct future research.
- The second part of this thesis will research a novel intervention which could be used to reduce infection-related mortality (IRM) a significant cause of cancer deaths that are not related to the disease.

1.2 Classification of death

1.2.1 The diagnosis and management of childhood cancers

The management of cancers involves a multi-disciplinary team (MDT) approach including haematologists, oncologists, surgeons and other allied health-care professionals.

Types of management can be broadly grouped into 2 components:

- Treatment directed at cancer (with or without curative intent), which may include the use of surgery, chemotherapy, immunotherapy and radiotherapy

- Treatments to support patients through interventions, including the management of toxicities, nutritional, psychological, and spiritual support.

Most patients are treated with curative intent, despite a predicted ‘poor’ outcome. For example, only 20% of children diagnosed with a metastatic rhabdomyosarcoma will survive longer than 5 years (12). Oncologists typically commence potentially curative treatment knowing that 80% of children treated will not survive.

Sometimes, a decision to proceed with non-curative treatment occurs at the time of diagnosis. For example, only 40% of children diagnosed with a diffuse intrinsic pontine glioma (DIPG) of the brain stem will survive longer than one year, and only 10% survive longer than 3 years (13). Patients with this diagnosis receive 6 weeks of radiotherapy. This treatment helps relieve symptoms and may prolong survival but will not cure the child.

Other non-curative treatments may involve the use of certain chemotherapies. For example, low-dose oral etoposide may be delivered with the aim of reducing symptoms and prolonging and improving quality of life in children with relapsed sarcoma (14).

All children diagnosed with cancer will require interventions to manage complications of therapies delivered (known as supportive care). Management may involve preventative strategies (e.g. the delivery of co-trimoxazole in immunosuppressed children diagnosed with ALL to prevent *Pneumocystis jiroveci*), interventional strategies (e.g. the use of platelets for a patient who has developed thrombocytopenia as a direct consequence of bone marrow suppression caused by chemotherapy), or treatment for long term effects (for example psychological therapy in a young adult who received cancer treatment as a child). Other supportive care interventions may include:

- Management of oncological emergencies: e.g. treating a child who has developed tumour lysis syndrome following a diagnosis of ALL,
- Care of central venous access devices,
- Management of infiltration and extravasation: e.g. chemotherapy accidentally delivered to subcutaneous tissue due to a faulty central venous catheter,
- Nutritional intervention: for example, delivery of enteral feeds via a nasogastric tube for excessive weight loss,
- Management of mucositis: e.g. delivery of pain relief for oral mucositis (damage to the mucosal barrier of the gastrointestinal system),
- Management of nausea and vomiting: e.g. use of antiemetics to prevent nausea and vomiting when delivering highly emetogenic chemotherapy drugs,

- Management of fluids and electrolytes: e.g. delivery of potassium supplements to treat hypokalaemia resulting from the development of chemotherapy-induced diarrhoea,
- Management of late effects: e.g. endocrine support following cranial radiotherapy,
- Social and financial support.

Curative strategies and supportive care implemented has resulted in improved survival (15). Further improvements may come from focussing on why children with cancer die; understanding which deaths are due to a failure of the anti-cancer therapy, and which are from the toxicities of therapy.

1.2.2 Causes of death in children diagnosed with cancers

The second most common cause of death in children globally, is cancer (13). Two hundred and fifty-seven children died of cancers in the UK between 2012-2014. This accounted for 23 cancer deaths per million children under the age of 14 (15). Twenty-five per cent of all cancers classified as CNS tumours were diagnosed in children and contributed to 35% of deaths by the end of 2016, whilst conversely, 31% of children were diagnosed with leukaemias, and these accounted for 23% of deaths (16). Three per cent of deaths in children diagnosed with CNS tumours had infection listed as one of the causes of deaths whilst twenty-seven per cent of children who died from leukaemias had infection listed as one of the causes of death. The UKALL2003 trial which occurred between 2003 and 2011 reported 249 deaths, of which 75 were due to infection-related mortality (IRM) (17). These findings highlight the burden of infection-related mortality particularly in malignant haematological conditions (16)

1.2.3 Classification of death

The increasing success of cancer-directed interventions due to increasing intensity is associated with a higher number of deaths due to toxicities arising from or complications of the therapies delivered (18). As previously described, death due to cancer is termed ‘disease-related’ or ‘disease-progression’. Death not directly due to cancer has been termed ‘treatment-related mortality’ (TRM). Causes of TRM deaths include infection, bleeding, and organ dysfunction (11). Because strategies are required to address disease-related and TRM differently, it is incumbent to attribute the cause of death correctly.

1.2.3.1 Case study: Disease-progression

An 8-year child diagnosed with a diffuse intrinsic pontine glioma receives 6 weeks of conventional radiotherapy. Whilst this child initially appears to improve, after a few

months, their initial presenting symptoms worsen. The child becomes increasingly drowsy and unresponsive. Because of tumour progression and its impact on the brain stem, the child subsequently dies.

1.2.3.2 Case study: TRM

An 4-year old child diagnosed with B-cell ALL has commenced induction therapy. This includes the delivery of dexamethasone, intrathecal methotrexate, vincristine and asparaginase. A bone marrow aspirate performed 8 days after the start of treatment demonstrates an excellent response to treatment with no evidence of disease. On day 15 of induction therapy, the child becomes febrile. A full blood count reveals the child is neutropenic. Despite the delivery of intravenous antibiotics, the child develops septic shock and subsequently dies.

The delivery of dexamethasone in combination with vincristine, asparaginase and intrathecal methotrexate resulted in bone marrow suppression exposing the child to overwhelming infection. This, alongside other factors, including initial bone marrow suppression due to disease, contributed to the cause of death; this death would be classified as TRM.

1.2.4 Reporting of TRM

As previously highlighted, TRM is still a significant cause of death in children diagnosed with cancer. Infection, particularly in children diagnosed with malignant haematological conditions, is thought to be one of the leading causes of TRM and is termed infection-related mortality (IRM).

However, despite this systematic reviews by Ethier et al, and Thai Tran et al (19, 20) highlighted a paucity of reporting of TRM. The study by Ethier et al reported that only 6.3% of the included studies examined reported the definitions or incidence of TRM, whilst the study by Thai Tran et al reported that only 16% of 64 identified studies reported TRM. These studies demonstrate how a lack of consistent reporting has made analysis and comparison of TRM and IRM difficult. Improving the harmonisation of definitions and recording of TRM will enable more reproducible reporting of causes of death as well as comparisons between trials.

1.2.5 Justification for the identification of TRM and disease-progression

In order to focus research efforts to improve survival further, knowing why children with cancer die; understanding where the deaths are through a failure of the anti-cancer therapy, and where they are from the toxicities of therapy, may allow us to direct research to the areas of highest need.

Diagnostic groups identifying disease-progression as a significant cause of death would benefit from strategies and research focused on improving curative outcome. For example, a study undertaken by Loeffan et al reported that out of 267 patients diagnosed with brain tumours 2.1% had died from TRM whilst 28.8% had died from disease-progression. This study highlights how pertinent it is to focus research on curative intent rather than on supportive care strategies for those diagnosed with CNS tumours (21).

Conversely, conditions identifying TRM as a significant cause of death would require strategies and research that would enable improved supportive care. For example, TRM is a significant cause of death in children with standard-risk ALL, despite its better overall survival (22). A significant number of TRM deaths identified are recognised as IRM. This highlights the need to focus on research and strategies on improving supportive care (for example, preventing infections).

To improve survival and reduce TRM and IRM in children with cancer the review of the literature undertaken for this thesis has highlighted the need for a uniform definition of TRM and classification of death in children with cancer which could be initially applied in the UK and high-income countries. A new classification system was developed and validated by Alexander et al in 2015 (23) in Toronto, Canada. However, this system had not been validated outside the centre it was developed.

1.3 Infection-related mortality

1.3.1 Infection, mucositis and probiotics

As previously introduced infection is a well-recognised cause of treatment-related mortality that occurs as a direct consequence of interventions delivered. The relationship of gastrointestinal mucositis, the breakdown and inflammation of the gastrointestinal lining is also known, but there is a lack of strategies to prevent infection that may develop from mucositis.

This section will, therefore, introduce and explore the relationship between infection mucositis and the gastrointestinal microbiome. It will then introduce the use of probiotics. Probiotics have previously been investigated in various paediatric conditions affecting the gastrointestinal system, but there is a lack of research investigating its use in children with cancer. The final part of this section then presents and justifies the need to investigate the use of probiotics in cancer therapy as a novel intervention to prevent and reduce mucositis and infections.

1.3.2 Infection

Infection is a leading cause of morbidity in TRM and poses a significant risk in patients diagnosed with malignant haematological conditions (16). Bone marrow suppression and damage to the mucosal barrier of the gastrointestinal system may occur following delivery of chemotherapy, immunotherapy or radiotherapy, and may also occur in bone marrow disease (17), affecting the child's ability to fight infection.

The UKALL 2003 trial reported 117 TRM deaths out of 3126 enrolled patients. Of these deaths, 64.1% were attributed to infection, constituting 2.4% of all included patients (18). In the USA, 63 (6.9%) out of 901 enrolled patients on the BFM-93 and AML-BFM 98 USA trials died of infection (16). This significant burden of death prompted the National Institute for Health and Care Excellence (NICE) to create national guidelines on the management of febrile neutropenia (19). Key recommendations include delivery of a beta-lactam monotherapy (e.g. piperacillin/tazobactam) within one hour of presenting with a temperature >38^{o}C and confirmed or suspected neutrophil count of less than 0.5 x10^{9}/L. Emerging concerns regarding the use of antibiotics and evolving microbial resistances are resulting in considerations of other preventative and treatment strategies and have, therefore prompting further exploration.

1.3.3 Mucositis

Mucositis is the inflammation and ulceration of the gastrointestinal mucosal lining that can occur in children diagnosed with cancer. It may be caused by radiotherapy or cytotoxic agents that affect DNA synthesis (particularly S-phase specific agents including cytarabine, methotrexate, actinomycin D, cisplatin, doxorubicin, daunorubicin, etoposide and mitoxantrone) (24).

Mucositis can occur in any part of the gastrointestinal system from the mouth to the anus. Symptoms range from mild erythema to widespread ulceration. Development of mucositis can, therefore, cause pain, nausea, malabsorption, malnutrition, diarrhoea, and increased risk of local and systemic infections (25, 26).

The Sonis hypothesis proposes 5 stages in the pathogenesis of mucositis:

1) Radiotherapy or cytotoxic exposure to the mucosal lining resulting in DNA damage and release of free radicals.

2) Activation of transcription factors, which results in the upregulation of proinflammatory cytokines causing mucosal destruction.

3) Signal amplification, which may exacerbate or prolong mucosal injury.

4) Ulceration.

5) Healing and gradual restoration of the flora (27).

The gold standard for the diagnosis of mucositis is by biopsy of the gastrointestinal mucosal lining. However, children typically require an endoscopy under general anaesthesia for this investigation, which is associated with significant risk, mainly as children are immunosuppressed and susceptible to complications, including severe infections and bleeding. Therefore, children with suspected mucositis are diagnosed clinically and graded according to the severity of reported symptoms using validated assessment scales such as the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE) (28) and the WHO guidelines for adverse events reporting (29, 30) summarised in table 1.

Table 1: Summary of sources and how mucositis is graded 0 (no symptoms) to grade 5 (death related to mucositis toxicity

Name of scale and source	Grade 0 (None)	Grade 1 (Mild)	Grade 2 (Moderate)	Grade 3 (Severe)	Grade 4 (Life threatening)	Grade 5 (Death)
Mucositis scale, CTCAE version v5.0 (31)	-	Asymptomatic or mild symptoms; intervention not indicated	Moderate pain; not interfering with oral intake; modified diet	Severe pain; interfering with oral intake	Life-threatening consequences: urgent intervention indicated	Death related to toxicity
Oral Mucositis Scale, World Health Organization (32)	None	Oral soreness and erythema	Oral erythema and ulcers: solid diet tolerated	Oral ulcers; liquid diet only	Oral alimentation possible	--
Acute Radiation Morbidity Scoring Criteria, Radiation Therapy Oncology Group (33)	No change over baseline	Injection; may experience mild pain not requiring analgesia	Patch mucositis that may produce inflammatory serosanguinitis discharge: may experience moderate pain	Confluent fibrinous mucositis; may include severe pain requiring narcotic	Ulceration, haemorrhage or necrosis	--

1.3.4 The relationship between mucositis and infection

The relationship between mucositis and febrile neutropenia is recognised, and the term ‘febrile mucositis’ is increasingly used (34). Patients with mucositis are believed to be most vulnerable to bacterial translocation from the gastrointestinal tract during stage 4 of the Sonis hypothesis, following damage to the epithelial lining of the mucosa and inflammatory amplification (35).

Studies have demonstrated the relationship between mucositis and febrile neutropenia (36). Mucosal damage which can occur following treatment with certain drugs (e.g. methotrexate),acts as a portal for the pathogen to enter the body. This, alongside neutropenia and immunosuppression, leaves the child in a particularly vulnerable state and at an increased risk of developing bacteraemia and sepsis. A study of adult patients diagnosed with multiple myeloma or non-Hodgkin’s lymphoma demonstrated a higher incidence of fever with severe mucositis when compared to those with less severe or no mucositis (68% vs 47%, difference 21%, p=0.004) (26). This relationship has consequently resulted in the term ‘febrile mucositis’ to reflect the different causes of fever in mucositis is now being used (25). However, studies investigating mucositis and infection in paediatric cancer patients are limited. A review of guidelines investigating the management of oral mucositis in children undergoing stem cell transplantation highlighted the epidemiology of mucositis is poorly understood, and that further observational studies and consensus-based approaches are required to understand and to develop appropriate risk stratification tools. It also stated further studies are required to investigate preventative measures for the development of mucositis (21).

1.4 The prevention and management of mucositis

Management of mucositis involves treatment once symptoms have developed. This includes the use of analgesia, loperamide to reduce diarrhoea, and delivery of nutrition using both enteral and parental routes.

Currently, there are no widespread preventative interventions for mucositis. Therapeutic strategies to manage mucositis include supportive strategies previously described alongside the delivery of antibiotics for potential severe infection.

Research into strategies to prevent febrile mucositis has been undertaken to investigate the use of probiotics to prevent or reduce mucositis in people with cancer. (31).

1.4.1 Probiotics

Probiotics according to the World Health Organisation and United Nations Food and Agriculture Organization (FAO) are defined as “live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host” (37). The most common strains used in probiotics belong to the genera *Lactococcus* and *bifidobacterium* (38). Health benefits attributed to probiotics include improved gastrointestinal flora, reduction in serum cholesterol, prevention of cancer, and reduced incidence of irritable bowel diarrhoeas (39).

Systematic reviews and meta-analyses have demonstrated potential benefits of the use of probiotics in a range of conditions. A review by Zhang et al (40) suggested administering probiotics prenatally to pregnant mothers and postnatally to children could reduce the risk of atopy (risk ratio (RR) 0.78, 95% confidence interval (CI) 0.57-0.89). A review by Aceti et al (41) states probiotics have an overall preventative effect for necrotizing enterocolitis (NEC) in preterm infants. They demonstrated probiotics prevented NEC in very-low-birth infants (RR 0.47, 95 % CI 0.37-0.62). There have been numerous systematic reviews published investigating the use of probiotics for gastrointestinal symptoms. A Cochrane systematic review by Goldenberg et al (42) concluded there was moderate-quality evidence suggesting probiotics confer a protective effect in preventing antibiotic-associated diarrhoea (AAD) in children (RR 0.46, 95 CI 035-0.61). Nevertheless, they noted the reporting of serious adverse events (SAE) in debilitated or immune-compromised children with underlying risk factors, including the development of probiotic associated infections, particularly from central venous catheter use. They recommended probiotic use should be avoided in paediatric populations at risk of adverse events. Another review by Szajewska and Kolodiej (43) suggested the probiotic *Lactobacillus rhamnosus GG* reduced the risk of AAD in adult and paediatric patients (RR 0.49, 95% CI 0.29-0.83). However, subgroup analysis revealed a risk reduction in only paediatric participants (RR 0.48, 95% CI 0.26-0.89). For adults, risk reduction occurred only in those receiving antibiotics as part of their *Helicobacter pylori* eradication therapy (RR 0.26, 95% CI 0.11-0.59). However, the quality of the included studies was recorded as moderate to low.

Gastrointestinal symptoms can occur in cancer patients receiving treatments. Chemotherapy and radiotherapy-induced diarrhoea is typically reported as an adverse event and is associated with fluorouracil, capecitabine, and irinotecan-based cancer regimes. It is estimated 20-45% of all chemotherapy patients experience severe diarrhoea (30). Radiotherapy is believed to potentially alter bacterial flora and affect the intestinal motility and vascular permeability of mucosal cells (44). Chemotherapy is thought to alter the composition of intestinal flora and therefore affect the metabolism of

intestinal enzymes vital for gut integrity. Changes to the gut flora may impact the gut defence barrier, immune function, and absorption of vital nutrients (45). Radiotherapy or chemotherapy-induced diarrhoea may interrupt or even stop treatment, impair the quality of life and prolong hospital stay of patients with cancer, potentially increasing health economic burdens too (46).

There has been interest in the role of probiotics in chemotherapy and radiotherapy associated diarrhoea. A previous systematic review and meta-analysis investigating the efficacy and safety of use of probiotics in people with cancer by Redman et al was published in 2014 (47). It proposed probiotics may reduce the severity and frequency of diarrhoea in patients with cancer following a review of 11 randomised-controlled trials (RCTs). Meta-analysis of 4 RCTs investigating the frequency of CTC grade >2 diarrhoea found that participants receiving probiotics showed a significant reduction in frequency when compared to the control group (OR 0.32, 95% confidence interval (CI) 0.13-0.79), and this may also have been the case with grade > 3 diarrhoea (OR 0.72, 95% CI 0.41-1.25). It suggested that in the probiotic groups soft/ semi-solid stools may occur more commonly (OR 0.46, 05% CI 0.04-5.64) and reduce the need for anti-diarrhoeal medicine (OR 0.63, 95% CI 0.13-0.79). Safety analysis of 17 studies and 1530 cancer patients revealed 105 adverse events (AE) in 756 people consuming probiotics and 145 AE in 774 people not consuming probiotics. Adverse events included bacteraemia/ fungaemias, infections, gastrointestinal symptoms, high blood pressure, and raised intracranial pressure. Five case reports of the 756 cases describing the consumption of probiotics reported bacteraemia/ fungaemia/ blood culture growth. Whilst these findings are encouraging it also highlights the need to re-assess the safety of probiotics in people with cancer.

Because probiotics contain bacteria which are believed to modify the gastrointestinal microbiome its relationship with the human microbiome and cancer therapy is now explored further.

1.5 The Human Microbiome

Humans are inhabited by a large number of microorganisms, and it is estimated that approximately 37 trillion cells inhabit the human microbiome. The ratio of microbial to human cells is reported to be 3:1 (48).

Microorganisms present in the human microbiome include bacteria, viruses, fungi and protists. Microorganisms residing within the microbiome have different functions (49). The human microbiome incorporates all microorganisms residing within or on any

human tissue or biofluid such as the skin, gastrointestinal system, uterus, and seminal fluid.

For the purpose of this thesis, the term ‘microbiome’ will refer to the gastrointestinal (gut) microbiome; and the gastrointestinal system will be the focus for the prevention and reduction of mucositis and infection.

1.5.1 The microbiome in infancy and childhood

Previously, it was hypothesised that the foetus is sterile, that colonisation of the microbiome occurs during delivery and birth, and that the mode of delivery (vaginal vs caesarian section) is a crucial modifiable factor impacting the development of the gut microbiome (50). However, recent studies have proposed that microorganisms colonise the amniotic fluid, umbilical blood cord and placenta, suggesting that colonisation of the infant occurs in utero (51, 52).

It is believed the presence of a healthy microbiome in term infants in the absence of infection or inflammation supports the proposition that not only do microorganisms colonise the foetus prior to delivery but that they may also contribute to the physiological development of the healthy foetus (53, 54).

During the first year of life, it was hypothesized that the gut microbiome changes from one representing maternal influences in utero to one consistent with that found in adults (50),although how this takes place is not fully understood. Some studies have reported *Bifidobacteria* bacteria are the most prevalent organisms of the gastrointestinal microbiota in breast-fed infants (55, 56) whilst other studies report they are only present in a small proportion of infants (57). One study reported a smaller proportion of *Bifidobacteria* bacteria and a higher proportion of aerobic bacteria in the gastrointestinal microbiota of formula-fed infants compared to infants who were breastfed (58). Other studies reported no differences (59).

An older study by Bezirtzoglou E. et al (60) proposed that at birth, the microbiota is typically aerobic and the most prevalent bacteria is Enterobacteriaceae phylum. Shortly after birth, the gastrointestinal microbiome becomes anaerobic, resulting in the growth of bacteria such as *Bifidobacterium* - thought to be the dominant bacterium genus in the first months of life. During weaning and introduction of solid food, a more adult-like microbiome develops between the ages of six months and one year, and the gut microbiome is subsequently dominated by *Firmicutes* and *Bacteroidetes* (60).

Dysbiosis (the disruption of the microbiome) in infancy and childhood is believed to be associated with an increased risk of immunological diseases such as asthma, type 1

diabetes, and celiac disease and metabolic diseases, e.g. obesity and type 2 diabetes (50).

Scientists have also been interested in the state of the microbiome before, during and following a cancer diagnosis. In recent years it has been proposed that changes to the microbiome can influence the development of toxicity (i.e. infection) and response to interventions (61).

1.5.2 The microbiome and its impact on cancer therapy

The term pharmacomicrobiomics is used to describe how the microbiota can affect drug metabolism and toxicity. Pharmacomicrobiomics is believed to determine the toxicity versus the efficacy of chemotherapy in different individuals (53).

A ‘balanced’ microbiome is believed to enhance therapeutic effects and reduce toxicity by manipulating how an individual responds to chemotherapy regimens and is based on an evaluation of the individual’s microbiome (62).

It is believed the gut microbiota impacts an individual’s response to chemotherapy through its relationship with the immune system (63). The microbiota is thought to contribute to the metabolism of chemotherapy and production of toxic metabolites, therefore altering the microenvironment and indirectly impacting how an individual metabolises chemotherapy. One study proposed the bacterial metabolism of bacterial vitamin B6, B9, and ribonucleotide can strengthen or weaken the effects of the chemotherapy drug 5-fluorouracil (5-FU) (64). A further study proposed disruption of bacterial deoxynucleotide pools can amplify 5-FU-induced autophagy and apoptosis (65).

The gut microbiota is impacted by multiple factors during chemotherapy. This includes diet, surgical intervention, supportive care interventions (i.e. antibiotics), and chemotherapy. A negative impact can result in dysbiosis, which can impair the symbiotic relationship of the microbiota and the individual, causing adverse side effects (i.e. diarrhoea) and thereby weakening the efficacy of the chemotherapeutic intervention (66).

The microbiota can also influence the host’s response to chemotherapy through modulation of the immune system. It is believed the interaction of the adaptive and innate immune system and the gut microbiota can regulate immunomodulation (66). Chemotherapy is thought to cause bacterial translocation through damage to the gastrointestinal mucosal epithelium, which causes systemic infections and exposure to pathogens and leads to priming of the adaptive immune system, thereby impacting how an individual responds to chemotherapy (67, 68).

It has also been proposed bacterial translocation and T-helper 17 cell activation may increase the efficacy of cyclophosphamide in mice with a 'healthy' gut microbiota when compared to mice raised in a sterile environment (69). Mice with depletion of gram-positive bacteria (i.e. commensal bacteria) due to vancomycin were found to have a reduced therapeutic response to cyclophosphamide when compared to the control group [(70). Intraluminal myeloid cell activation is thought to enhance the action of oxaliplatin (71). However, the impact of the human gut microbiota on cyclophosphamide has not been investigated.

A study by Zieglar et al 2019 (72) evaluated the impact of a prophylactic antibiotic (levofloxacin) to prevent febrile neutropenia when compared to broad-spectrum beta-lactam (BSBL) antibiotics on the gut microbiome in patients with haematological malignancies (72). In sixty patients the gut microbiome of patients with BSBL exposure had significantly reduced diversity when compared to those without (median, interquartile range (IQR), 3.28 [1.73 to 3.71] vs 3.73 [3.14 to 4.31]; p = 0.01). Patients receiving levofloxacin were found to have increased gut microbiota diversity when compared to those not receiving it (median IQR, 3.83 [3.32 to 4.36] vs 3.32 [2.35 to 4.02]; p = 0.03). Levofloxacin exposure was also associated with a trend towards a lower risk of the dominance of non-Bacteroidetes genera compared to those without levofloxacin exposure (3 [14%] vs 15 [38%]; p = 0.051). All this suggests gut microbiome interacts with chemotherapy delivered; supportive care strategy deliveries could lessen toxicity experienced by a patient in current and future courses of chemotherapy. There is a need to investigate how the gut microbiota could be manipulated to reduce infections and therefore, potentially reduce mortality (72).

1.6 Summary

In summary, the survival of children diagnosed with cancer has improved during recent decades. Earlier diagnoses, improved curative treatment and supportive care strategies have reduced overall mortality. However, death attributed to treatment-related mortality, particularly infection-related mortality from increased toxicity of intensive treatment strategies, is still a significant concern.

Treatment-related mortality (TRM) is poorly defined and reported in studies. Increased accuracy and reporting of TRM and infection-related mortality (IRM) will harmonise results, enabling better comparisons of clinical trials.

Despite the recognised relationship between mucositis and febrile neutropenia, there are no widely used preventative or therapeutic interventions for febrile mucositis. Exploration of possible strategies may result in reduced TRM due to infections.

This chapter has highlighted and presented two critical areas of research within this topic that require further exploration:

1) The need for a uniform definition of TRM and disease-progression for the classification of death in children with cancer, applicable in high-income countries.
2) The need for preventative and treatment strategies to reduce TRM in children diagnosed with cancers, potentially through the modification of the microbiome to reduce mucositis, bacterial translocation and bloodstream infection.

As infection is a significant and leading cause of TRM, this thesis will focus on the reporting of classification of death and on the use of probiotics to reduce and prevent infection and mucositis in children with cancer.

Therefore, the research undertaken for this PhD intends to:

- Investigate and validate a newly developed definition of TRM through the use of a classification tool and cause of death attribution system
- Investigate the use of probiotics in gastrointestinal mucositis by updating a systematic review and meta-analysis published in 2012 (47)
- Use the findings of the review to conduct a feasibility study investigating the use of probiotics in children with cancer at who are at risk of or who have previously developed mucositis.

2 Validation of a classification system for treatment-related mortality in children with cancer

2.1 Introduction

The previous chapter introduced key concepts around childhood cancers, including mortality, classification of death, and how supportive care strategies can be used to prevent and reduce morbidity and mortality. It demonstrated that despite advances in medical research and in the management of patients, death is still a concerning and significant outcome in children diagnosed with cancer. Treatment-related mortality (TRM) is one major cause of death, but it is poorly defined and understood.

As previously described varying definitions of TRM are used; identifying the cause of death (treatment-related mortality vs disease-progression) is necessary to identify where to focus research, strategies and interventions. Systematic reviews performed by Ethier MC et al (20) and Tran Thet al (19) identified significant heterogeneity in TRM definitions used in randomised therapeutic trials. This inspired a global collaboration led by Lillian Sung from Toronto in Canada to develop and validate a consensus-based classification tool for ascribing death as a TRM (figure 1) alongside further specifics of causes of death (table 2) (23).

The tool is intended for use by clinical research assistants (CRA), a term used to describe non-medically qualified professionals who work with clinical trial data capture and entry. Etiological categories, e.g. infection or haemorrhage, were derived from the International Statistical Classification of Diseases and Related Health Problems (ICD)-10 and Common Terminology Criteria for Adverse Events (CTCAE) v4.0. The classification system developed was validated by a retrospective case review of 30 cancer patients who died between 2003 and 2012 by 2 independent blinded medical and CRA reviewers. Reliability for the TRM classification was deemed to be almost perfect between the medical and CRA reviewers who had been involved in developing the system (kappa=0.92, 95% confidence interval 0.78-1.00).

An ideal classification system for treatment-related mortality should be applicable across different countries, treatment protocols, and health care settings. It is therefore essential to attempt to further validate the proposed classification tool and attribution system in a different treatment centre. Therefore, this chapter describes the efforts to further validate the consensus-based definition of treatment-related mortality and cause-of-death attribution system at Leeds Teaching Hospital, UK, with a group of individuals who were not involved in the development of the system.

This study aimed to evaluate the reliability of the newly developed consensus-based definition of TRM and explore the use of the cause-of-death attribution system at a regional paediatric oncology centre in Leeds, England.

2.2 Methods

This study was approved by the University of Leeds School of Medicine Ethics committee (Ref: MREC15-118) (appendix 1.1) and did not require NHS ethics approval. Eligible patient records were those of patients treated for malignancy or who underwent a haematopoietic stem cell transplant (HSCT) for a non-malignant diagnosis at Leeds Children's Hospital (Leeds, UK) while aged 18 years or younger at diagnosis. Five cases were excluded as patients had died following relapse after the age of 18, or the medical records could not be located. All included patients died between 2014 and 2016. Data from the clinical records were anonymised and presented in a different random order for each assessor. Thirty patient records were included. Each set of records was presented twice, once with information from 2 weeks prior to death, once with the information extending back to 4 weeks prior to death, leading to a total of 60 assessments being made. Four participants were identified to review the case notes; the two CRAs were a data analyst (AF) and research nurse (JT), and the two senior clinicians were a consultant paediatric oncologist and consultant paediatric haematologist (AG and SK).

The study was undertaken on a single afternoon. After reading a participant information leaflet (appendix 1.2) and signing a consent form (appendix 1.3), participants received a 10-minute educational presentation explaining how to use the system, and how the study would be undertaken. The reviewers then independently classified each death according to the algorithm (fig. 1). For cases assessed as TRM, the reviewers were asked to apply the cause-of-death attribution system (table 2) to identify a primary cause of death. Following the completion of the assessments, a moderated group discussion was undertaken with notes recorded by two facilitators being used to supplement the themes of the discussion.

Inter-relater reliability was assessed using the Kappa statistic (k). Criterion validity was assessed by assuming classification by the Consultants as the gold standard. Group consensus classification between and within the CRAs and Consultant group was evaluated using the Cohen's kappa statistic, and across all individuals using the Fleiss' kappa statistic. The strength of agreement was defined as slight (0.00-0.20), fair (0.21-0.40), moderate (0.41-0.60), good (0.61-0.80) and very good (0.81-1.00) (73). A numerical code was used to combine agreement/disagreement between the individual consultants (TRM was recorded as "0", and non-TRM outcomes were recorded as "1"

in Excel). If individual disagreement was noted when calculating inter-relater reliability between the CRAs and consultants, the outcome was recorded as “2”. Based on the previously published study (23), we decided to include 30 cases for analysis. A sample size of 27 deaths determined whether k was good (i.e., ≥0.61), with a power of 0.80, and two-sided α of 0.05, and assuming that treatment-related mortality accounted for 20% of deaths (6). A further 30 cases would be reviewed if validity was inadequate (defined a priori as k<0.6). Calculation of the k statistic was completed using the R studio irr package, and bootstrap with 2000 iterations was used to calculate 95% confidence intervals (74). Comparison of the cause of death was qualitative, and to provide further insight reviewers participated in a discussion of the use of the algorithm and cause of death attribution system.

2.2.1 Inter-relater reliability

This study was designed to include reviewers of different job roles to demonstrate that the TRM classification tool can be used by individuals with varying clinical experience and skills. Analysis of results, therefore compared agreement of outcomes recorded using the algorithm in figure 1. Reliability of the study was dependent on the amount of ‘disagreement’ or error that occurred. The extent of agreement between the reviewers was termed ‘interrater reliability’. Inter-relater reliability of the CRAs and of the consultants was assessed using the Kappa statistic (k). This is a statistic that measures the degree of agreement between independent reviewers. It is more accurate and reliable than percentage agreement calculation of reviewers as it factors in the proportion of agreement which may occur due to chance.

Calculation of the k statistic was completed using the R studio irr package and bootstrap method (see below) (74). The relevant software packages “lpsolve” and “irr” required to calculate k statistics were downloaded. Cohen’s kappa (for two raters) and Fleiss kappa (an adapted Cohen’s kappa for 3 or more raters) formulas were used to calculate the appropriate k statistic (73). The Fliess kappa also allows each rater to rate different items, while Cohen's kappa assumes that both raters are rating identical items. Therefore, the fliess kappa should be be used when raters responsible for rating one subject are not assumed to be the same as those responsible for rating another (75).

As the irr package does not include methods to calculate confidence intervals, this had to be ‘bootstrapped’ into R studio.

2.2.2 Bootstrapping

“Statistical Bootstrapping” is a method used to calculate measures of estimation (for example, variance, confidence intervals and prediction errors) of a population from a

sample (76). As only one k statistic can be calculated from one study sample, a bootstrap sample is created using the sampling with replacement method (a result within the population can be used more than once). As an element can be repeated more than once it ensures that all unique outcomes are considered with resampling. This is then repeated a large number of times (typically 1000 or more), and an average k statistic is calculated for each bootstrap sample (also known as bootstrap estimates). Once a sufficient number of bootstrap estimates are calculated, the central limit theorem (77) can be applied to assume that the bootstrap estimates are reflective of the population. The distribution of bootstrap estimates is then used to calculate the measure of variance or in this case, confidence intervals. A code was imputed into R studio to resample the results of the study, and this was bootstrapped 2000 times to calculate the bootstrap estimates (95% confidence intervals).

2.2.3 Qualitative analysis

Comparison of the cause of death attribution system was qualitative. Following the review of the clinical records, reviewers then participated in a discussion of the use of the algorithm and cause of death attribution system.

Figure 1: Classification of TRM in children with cancer, taken from (23)

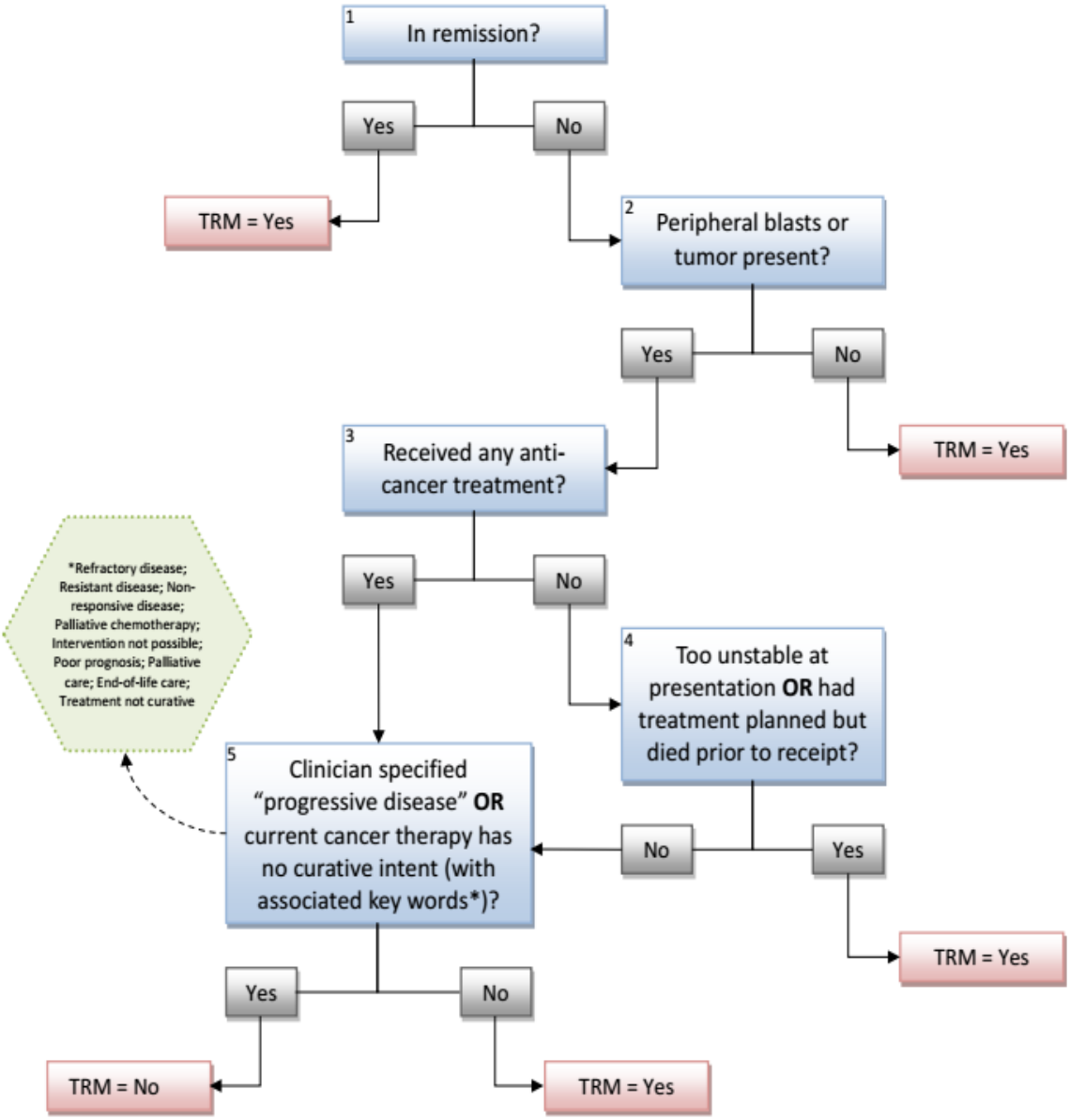


Table 2: Cause-of-death attribution system.

Cause-of-death	Probable cause of death	Possible cause of death
Infection	A.1 Clinically or radiographically documented infection with associated microbiologically documented organism	1. Clinically or radiographically documented infection without associated microbiologically documented organism.
Haemorrhage	B.1 Acute symptomatic intracranial haemorrhage shown by imaging or pathology. B.2 Acute symptomatic pulmonary haemorrhage shown by imaging or pathology. B.3 Acute symptomatic bleeding resulting in hypotension, urgent transfusion or fluid bolus.	2. Acute symptomatic pulmonary haemorrhage not shown by imaging or pathology
Thrombosis	C.1 Acute symptomatic intracranial thrombosis or embolism shown by imaging or pathology. C.2 Acute symptomatic pulmonary thrombosis or embolism shown by imaging or pathology. C.3 Acute symptomatic hepatic thrombosis or embolism shown by imaging or pathology.	3. Acute symptomatic pulmonary thrombosis or embolism not shown by imaging or pathology.
Cardiac	D.1 Acute symptomatic arrhythmia excluding sinus tachycardia or bradycardia shown by ECG. D.2 Acute symptomatic cardiac dysfunction defined by ECG, cardiac imaging or pathology.	4. Acute symptomatic arrhythmia excluding sinus tachycardia or bradycardia not shown by ECG.
Immunomediated	E.1 Acute allergic reaction, anaphylaxis with symptomatic bronchospasm, oedema, angioedema, or hypotension. E.2 Worsening symptomatic graft versus host disease. E.3 Acute symptomatic haemophagocytic lymphohistocytosis, macrophage activation syndrome or cytokine-release	5. Stable graft-versus-host disease.

Cause-of-death	Probable cause of death	Possible cause of death
	syndrome.	
Metabolic	F. Clinically diagnosed tumour lysis syndrome with cardiac arrhythmia, seizure, or creatinine concentrations greater than 3 times the ULN.	
CNS	G.1 Acute symptomatic CNS necrosis shown by imaging or pathology G.2 Acute symptomatic encephalopathy shown by imaging or electroencephalography G.2 Acute symptomatic hydrocephalus or raised intracranial pressure shown by imaging, pathology, or measurement of intracranial pressure G.4 Seizure lasting at least 30 minutes within 48 hours of death	6.1 Acute symptomatic CNS necrosis not shown by imaging or pathology 6.2 Acute symptomatic encephalopathy not shown by imaging or electroencephalography 6.2 Acute symptomatic hydrocephalus or raised intracranial pressure not shown by imaging, pathology, or measurement of intracranial pressure 6.4 Seizure between 5 and 30 minutes within 48 hours of death
Respiratory	H. Acute symptomatic respiratory distress with ventilator support	7. Acute symptomatic respiratory distress without ventilator support
Gastrointestinal system	I.1 Acute symptomatic bowel disease resulting in necrosis, obstruction or perforation shown by imaging or pathology I.2 Acute, clinically diagnosed hepatic dysfunction associated with conjugated bilirubin concentrations greater than 10 x ULN, ammonium concentrations greater than 2.5 x ULN, or international normalised greater than 2.5 times the ULN I.3 Acute, clinically diagnosed pancreatitis with haemorrhage, peritonitis, necrosis or haemodynamic instability (evidenced by hypotension, urgent transfusion, fluid bolus, or vasopressers	8.1 Acute symptomatic bowel disease resulting in necrosis, obstruction or perforation not shown by imaging or pathology 8.2 Acute, clinically diagnosed hepatic dysfunction associated with conjugated bilirubin concentrations greater than 1.5 and less than 10 x ULN, ammonium concentrations greater than 1.5 and less than 2.5 x ULN, or international normalised greater than 1.5 but less than 2.5 times the ULN
Renal system	J. Acute kidney injury with dialysis or renal replacement therapy (planned or received)	

Cause-of-death	Probable cause of death	Possible cause of death
External causes	K.1 Unintentional injury (e.g accident) K.2 Suicide K.3 Homicide	

Abbreviations

ULN: upper limit of normal

ECG: electrocardiography

CNS: central nervous system

2.3 Results

2.3.1 Summary of results

Twenty patients included in the review were diagnosed with solid tumours, and 10 were diagnosed with malignant haematological conditions (summarised in table 5). Thirty-three per cent of total deaths were classified as TRM. Ten per cent of patients diagnosed with solid tumours and 80% of patients diagnosed with malignant haematological conditions were classified as TRM. Reliability of classification was almost perfect between CRAs and consultants, with a k statistic of 0.86 (95% confidence interval 0.72-0.97). There was also almost perfect agreement between CRAs (k=0.96, 95% confidence interval 0.87-1.00) and consultants (k=0.85, 95% confidence interval 0.67-0.97), a summary of the results is shown in table 3. Table 6 summarises the percentage agreement and bias index**.** The CRAs disagreed only in one case, whilst the consultants disagreed on 2 cases. There were 3 disagreements when results of CRAs were compared with the consultant's decisions. This will be discussed further in this chapter.

2.3.2 Demographics

Age of identified patients ranged from less than 1 to 17 years, and 57% (17) were male. 67% (20) were diagnosed with solid tumours, and 33% (10) were diagnosed with malignant haematological conditions. Collectively, sarcomas were the most frequent solid tumour diagnosed (12 cases, 40% of total), followed by CNS tumours (9 cases, 30% of total). 27% (8 cases) of these patients had presented with metastatic disease at diagnosis. All patients with malignant haematological conditions were diagnosed with leukaemia. 40% (12 cases) of patients had either received a transplant or presented with relapsed disease. A summary is enclosed in table 5.

2.3.3 Classification of treatment-related mortality

Ten deaths (33%) were identified as TRM by at least one reviewer. Three (15%) of patients diagnosed with solid tumours and 80% (8 cases) of patients diagnosed with malignant haematological conditions were classified as TRM. Reliability of classification was very good between CRAs and consultants, with a k statistic of 0.86 (95% confidence interval 0.72-0.97, with disagreement on 3 deaths). There was also very good agreement between CRAs (k=0.96, 95% confidence interval 0.87-1.00, disagreement on one record) and between consultants (k=0.85, 95% confidence interval 0.67-0.97, disagreement on two deaths) (tables 3).

When the 2 and 4-week data were examined, there was a single difference between the assessments of each of the four assessors.

2.3.4 Cause of death attribution system

Table 5 summaries the diagnoses of those whose deaths were classified as TRM and the causes according to the cause of death attribution summarised in table 2.

All reviewers unanimously agreed on the cause of death of 3 cases (J, V, AA). Reviewers failed to agree on the primary cause of death in 7 cases (M, X, Y, Z, AB, AC and AD).

Infection was the most common cause of death attributed in 6 of the 10 patients with treatment-related mortality (V, X, Y, Z, AA and AB). However, reviewers failed to unanimously agree on the primary cause in 4 of these cases (X,Y, Z and AB).

The next most common cause of death attributed was haemorrhage (M, X, Y, AC), followed by immunomediated (J, AC, AD). Respiratory (Y,Z) and renal (AD) were other causes attributed.

Reviewers failed to agree on the cause of death attribution when there were multiple factors which contributed to the death of a patient; one example involved a patient diagnosed with standard-risk B-cell ALL who developed febrile neutropenia and gastrointestinal haemorrhage (case X). Reviewers struggled to attribute the cause of death for case AD (cause of death on death certification was recorded as multi-organ failure), and both CRAs did not list a cause.

2.3.5 Post-review discussion

The reviewers agreed that the TRM algorithm was straightforward to use and that it would be beneficial to have a standardised tool and attribution system to use in trials.

Questions highlighted in completing the review included how to address the patients who may die from TRM whilst receiving palliative care and particularly how the algorithm could be used as part of palliative care study.

In one particular case, a patient (case D) was taking palliative etoposide following a diagnosis of relapsed ALL and developed a febrile illness. Another question raised was how to define the end of treatment in cancer patients. This question arose because a child (case V) who had completed treatment for standard risk AML died of overwhelming pneumococcal septicaemia 6 months after the end of treatment. It is important to note the child had not been re-vaccinated at this point. Suggestions of 3 months after the end of treatment for standard patients, 12 months post-transplant for high-intensity therapies, or following revaccination were proposed. Another case the reviewers felt was important to highlight was how to classify the death of somebody who dies during surgical intervention. This question was raised following the case review of a patient (case M) who presented acutely with signs of raised intracranial

pressure and large mass noted on CT. The patient died on the operating table whilst receiving a surgical intervention.

The case the reviewers found most challenging to categorise involved a patient who died of multi-organ failure following an HSCT (AD). Both CRAs, in particular, felt it was difficult to attribute one organ system to the cause of death, and both independently decided not to attribute a cause of death.

Table 3: Summary of kappa statistic and 95% confidence intervals of independent reviewers, consultants, CRAs and between CRA and consultants for all total case reviews, 4 weeks and 2 weeks prior to death using the cause of death attribution system.

Inter-rater comparison	Total k (95% confidence interval)	4 weeks k (95% confidence interval)	2 weeks k (95% confidence interval)
Independent reviewers *	0.92 (0.83-0.98)	0.91 (0.76-1.00)	0.92 (0.79-1.00)
Consultants **	0.85 (0.67-0.97)	0.85 (0.59-1.00)	0.84 (0.59-1.00)
CRA **	0.96 (0.87-1.00)	0.85 (0.59-1.00)	0.85 (0.60-1.00)
CRA vs consultants**	0.86 (0.72-0.97)	0.87 (0.66-1.00)	0.86 (0.67-1.00)

*calculated using the Fleiss kappa statistic (between 4 reviewers).

**calculated using the Cohen's kappa statistic (between 2 reviewers or 2 groups).

Table 4: Diagnosis of included patients results of classification by reviewers

Case	Diagnosis	CRA 1	CRA 2	Consultant 1	Consultant 2
A	Germ cell CNS tumour	Not TRM	Not TRM	Not TRM	Not TRM
B	Diffuse intrinsic pontine glioma (DIPG)	Not TRM	Not TRM	Not TRM	Not TRM
C	Thalamic astrocytoma	Not TRM	Not TRM	Not TRM	Not TRM
D	Relapsed B-cell ALL	Not TRM	Not TRM	Not TRM	Not TRM
E	Spinal cord glioblastoma multiforme	Not TRM	Not TRM	Not TRM	Not TRM
F	Metastatic Ewing's sarcoma	Not TRM	Not TRM	Not TRM	Not TRM
G	Metastatic Ewing's sarcoma	Not TRM	Not TRM	Not TRM	Not TRM
H	Metastatic rhabdomyosarcoma	Not TRM	Not TRM	Not TRM	Not TRM
I	Philadelphia positive ALL	Not TRM	Not TRM	Not TRM	Not TRM
J	AML prior to HSCT	TRM	TRM	TRM	TRM
K	Metastatic rhabdomyosarcoma	Not TRM	Not TRM	Not TRM	Not TRM
L	Soft tissue myoepithelial carcinoma	Not TRM	Not TRM	Not TRM	Not TRM
M	ATRT	TRM	TRM	Not TRM	TRM
N	Metastatic Ewing's sarcoma	Not TRM	Not TRM	Not TRM	Not TRM
O	High risk neuroblastoma	Not TRM	Not TRM	Not TRM	Not TRM

P	Osteosarcoma	Not TRM	Not TRM	Not TRM	Not TRM
Q	DIPG	Not TRM	Not TRM	Not TRM	Not TRM
R	Osteosarcoma	Not TRM	Not TRM	Not TRM	Not TRM
S	High risk neuroblastoma	Not TRM	Not TRM	Not TRM	Not TRM
T	Malignant melanoma of the CNS	Not TRM	Not TRM	Not TRM	Not TRM
U	Metastatic Ewing's sarcoma	Not TRM	Not TRM	Not TRM	Not TRM
V	AML	TRM	TRM	TRM	TRM
W	Epithelial sarcoma	Not TRM	Not TRM	Not TRM	Not TRM
X	ALL	TRM	TRM	TRM	TRM
Y	T-cell ALL	TRM	TRM	TRM	TRM
Z	B-cell ALL	TRM	TRM	TRM	TRM
AA	B-cell ALL	TRM	TRM	TRM	TRM
AB	Ependymoma	TRM	TRM	Not TRM	TRM
AC	T-cell ALL	TRM	TRM	TRM	TRM
AD	B-cell ALL post HSCT	TRM	TRM	TRM	TRM

Abbreviations

CNS central nervous system **HSCT** haematopoietic stem cell transplantation (HSCT)

AML acute myeloid leukaemia

DIPG diffuse intrinsic pontine glioma

ALL acute lymphoblastic leukaemia **ATRT** atypical teratoid rhabdoid tumour

Table 5: Summary of the cause of death attribution by reviewers for deaths classified as TRM.

Bold font=probable causes of death

Case	Diagnosis	CRA 1	CRA 2	Consultant 1	Consultant 2
J	AML prior to HSCT	Immunomediated	Immunomediated	Immunomediated	Immunomediated
M	ATRT	Acute symptomatic intracranial haemorrhage	Acute symptomatic intracranial haemorrhage	NR	Acute symptomatic intracranial haemorrhage
V	AML	Infection	Infection	Infection	Infection
X	B-cell ALL	Acute symptomatic intracranial haemorrhage	Acute symptomatic intracranial haemorrhage	Infection	Infection
Y	T-cell ALL	Respiratory	Infection	Acute symptomatic intracranial haemorrhage	Infection
Z	B-cell ALL	Respiratory	Infection	Respiratory	Infection
AA	B-cell ALL	Infection	Infection	Infection	Infection
AB	Ependymoma	NR	Infection	NR	Infection
AC	T-cell ALL	Worsening symptomatic graft versus host disease.	Worsening symptomatic graft versus host disease	Worsening symptomatic graft versus host disease	Acute symptomatic pulmonary haemorrhage
AD	B-cell ALL post HSCT	“Unclear”	“Difficult case”	Worsening symptomatic graft versus host disease	Acute kidney injury

Table 6: Results of percentage agreement and bias index of reviewers.

	Result
Percentage agreement	93.3%
Bias index	0.03

2.4 Discussion

To the best of our knowledge, this study is the first revalidation of the standardised definition of treatment-related mortality and cause of death attribution system for paediatric cancer patients (23). It demonstrates that the system is reliable and establishes its validity in an alternative centre and health care system with different treatment protocols. It can be used after minimal training, with “very good” agreement between assessors irrespective of discipline (Fleiss kappa 0.92, 95% CI 0.83-0.98). The study confirms the observations of the development group and shows that information from two weeks prior to the death of a patient is sufficient to attribute death to either TRM or disease consistently.

2.4.1 Strengths of the study

Criterion validity and almost perfect agreement between CRAs and consultants were demonstrated using the k statistic. However, using a measure of inter-rater agreement as a measure of reliability should be done cautiously. The use of the k statistic with dichotomous outcomes (e.g. dead or alive) is more reliable when compared to the use of non-dichotomous outcomes which require finer discriminations and are subject to different interpretation, as was the case in this study. Reliability in cases which require more information can be improved with appropriate training. To increase the reliability of agreement in this study, a participation information leaflet (appendix 1.2) was produced along with a short presentation. Despite this attempt to increase reliability between reviewers, external factors can also influence decision making. This may include previous experience, level of clinical expertise, and interpretation of the training delivered.

2.4.2 Agreement

As previously discussed in the methods, use of the k statistic is believed to be more reliable than using percentage agreement, as the k statistic factors in the possibility of agreement occurring to chance (73). However, the presumed proportion of random agreement included in the analysis, assumptions about intra-rater independence, and other factors, may result in potential underestimation of agreement between reviewers. It is, therefore, useful to compare the results of the k statistic with the percentage agreement statistic. Whilst the percentage agreement statistic does not factor agreements occurring due to chance and may over-inflate the agreement; it is a useful statistic to use when raters have adequate knowledge and training and are less likely to guess decisions (78). The k statistic is useful when there is more likely to be confusion in decision making and therefore, potentially a more significant proportion of

agreements occurring due to chance. For this study, using both statistics gives greater credibility to the conclusions drawn.

Other inter-reliability agreements which could have been used include the intraclass correlation coefficient, person r, the contingency coefficient, and Krippendorff's alpha. However, the use of some of these agreement statistics may be difficult to interpret with non-exchangeable observers and may result in the extreme under or overestimation of inter-rater agreement (78).

2.4.3 Limitations of the study

Although consultants were considered gold-standard in this study, it was identified that even experienced clinicians might disagree on the cause of death when using the algorithm. Whilst the initial study [7] asked the consultants to discuss and resolve any differences in how each death was classified; this was not undertaken in this study as this was more reflective of how the classification tool would be used in a clinical setting. Consultants disagreed on the classification of death in two cases. This may have occurred due to the individual consultant's clinical experience or previous contact with the patients concerned. Even though the cases were anonymised and randomised, the consultants may have recognised the patient due to their involvement in the delivery of clinical care. The differences identified highlights how the TRM classification tool would never have perfect agreement between reviewers irrespective of experience, or clinical and scientific knowledge.

In this study, reviewers attributed death to one primary probable, or possible, cause. Whilst developing the study protocol, we decided to limit the number of causes of death for simplicity. However, reviewers found it challenging to identify only one cause of death, and to distinguish between probable and possible causes.

Since the development of this study, a standard operating procedure TRM web-based tool has been published (https://www.sungresearch.com/trm-training-manual/) and includes working examples. Use of this tool when delivering the training package should help clarify how to use the cause-of-death attribution system and minimise misunderstanding. Currently, the web-based tool is available in English. Having the tool available in other languages could potentially reduce confusion and improve harmonisation across clinical trials.

2.5 Other considerations

The dichotomous definitions (TRM and cancer-deaths) and semantic interpretations of the classification (what type of deaths are included under each definition) have been previously explored. What these definitions do not identify is a third group of deaths

that can occur in cancer patients; deaths due to non-cancer causes (or ‘other natural’). During the participant discussion after the study, the participants highlighted the death of patient not included in the study. This particular patient had completed treatment for Hodgkin’s lymphoma and attended the clinic for a routine follow up 3 months later. The family was involved in a road traffic accident on the way home, and the patient subsequently died in hospital. Whilst such a cause of death is uncommon, it is still not appropriately addressed by the use of the terms TRM and cancer-deaths. Under the current classification, this death would have been defined as TRM death, which is misleading. This could be addressed in a number of ways; firstly, by clearly defining the end of treatment in cancer patients or by adding a third type of death - ‘other natural’.

The occurrence of death due to natural causes, when compared to treatment-related deaths, will increase with time following the end of treatment. Not defining an end of treatment date when using the algorithm may contribute to over-inflation of deaths reported as TRM. The chosen definition would need to consider the probability of death occurring to natural causes or late effects of cancer therapies. Creating a third group could potentially reduce the over-reporting of TRM deaths and therefore increase the accuracy of the data. However, as previously discussed, this may complicate the use of the tool when implementing the system across the world in translation.

Other limitations of the tool include incorrect classification due to inaccurate or incomplete information documented in the medical records or due to use of different types of records (for e.g. computer-based vs handwritten records). Currently, the tool can only be implemented in high-income and possibly middle-income countries due to reduced availability of interventions, medical care and due to issues with follow up.

2.5.1 Cause of death attribution system

During the discussion, reviewers stated they felt the cause of death attribution was easy to follow. However, reviewers failed to agree on the cause of death in 6 of the 10 TRM episodes (X, Y, Z, AB, AC, AD) summarised in table 5. In 5 cases (X, Y, Z, AC, AD) death involved complications of multiple systems, and therefore reviewers were unable to agree on the primary cause of death. In 2 cases (M, AB) reviewers disagreed on the classification of death (TRM vs cancer-death). Case AD was felt to be a particularly difficult case for the reviewers, and both CRAs did not attribute a cause of death. Interpretation of the attribution system was felt to require more subjective interpretation when compared to the dichotomous decision making of the classification tool. Discrepancies may be attributed to a number of external factors including previous experience, clinical expertise and interpretation of clinical documentation of the reviewers.

Although the tool was developed with the intention of widespread use by CRAs of varying levels and experience, it may require users to meet specific requirements of expertise or experience. This study showed discordance between consultants (deemed gold standard for criterion validity). In this study, the consultants disagreed in 5 cases (M, Y, Z, AB, AD), whilst CRAs disagreed on 3 cases (Y, Z, AB). Differences between the consultants may be attributed to their qualification (consultant haematologist and consultant oncologist), which may result in differing views of supportive care, or previous involvement with some patients.

Reviewers' understanding and interpretation of the cause of death attribution system could possibly be improved further by dedicating more time and expanding in greater detail on the use of the cause of death attribution system. Another possibility involves potentially refining the cause of death attribution system. This could include having the option of listing multiple causes of death (with or without ranking) or refining the current options. For example, rather than having 'respiratory' and 'infection' as 2 separate entities 'respiratory infection' could be listed as a subcategory. However, this would increase the information in the cause of death attribution table, potentially making it more challenging to navigate and translatable to different health-care settings and languages.

2.5.2 Challenges and areas for future development

Our study highlighted specific challenges with the system as it currently exists, both with the classification of TRM and the attribution of a specific cause of death. Fundamentally, this approach defines deaths as either 'treatment-related' or 'cancer related'. This gives rise to a semantic challenge; "treatment-related mortality" implies that deaths that come under this term occur directly because of the therapies delivered. However, the classification system also classifies deaths occurring prior to the commencement of anti-cancer therapy, which are not directly attributable to cancer (for example, tumour lysis syndrome in high-count leukaemia) as cases of TRM. This clash of language and "common sense" may confuse users of the classification tool, for example, case M in which a patient presented acutely with signs of raised intracranial pressure and died on the operating table.

A more profound challenge to this system addressed the philosophical distinction between assigning deaths into one of two categories; cancer or treatment-related. There is a convincing argument that a third category of death should be attributable, "other non-cancer death", for those who die of an event or illness external to their malignancy. This problem is particularly evident if the current system is to be used after the completion of treatment. For example, a patient dies as a passenger in an air traffic accident 4 years after treatment for a localised Wilms tumour. Under the current

system, this death would be classified as TRM, even though the death would be unrelated to the child's cancer diagnosis.

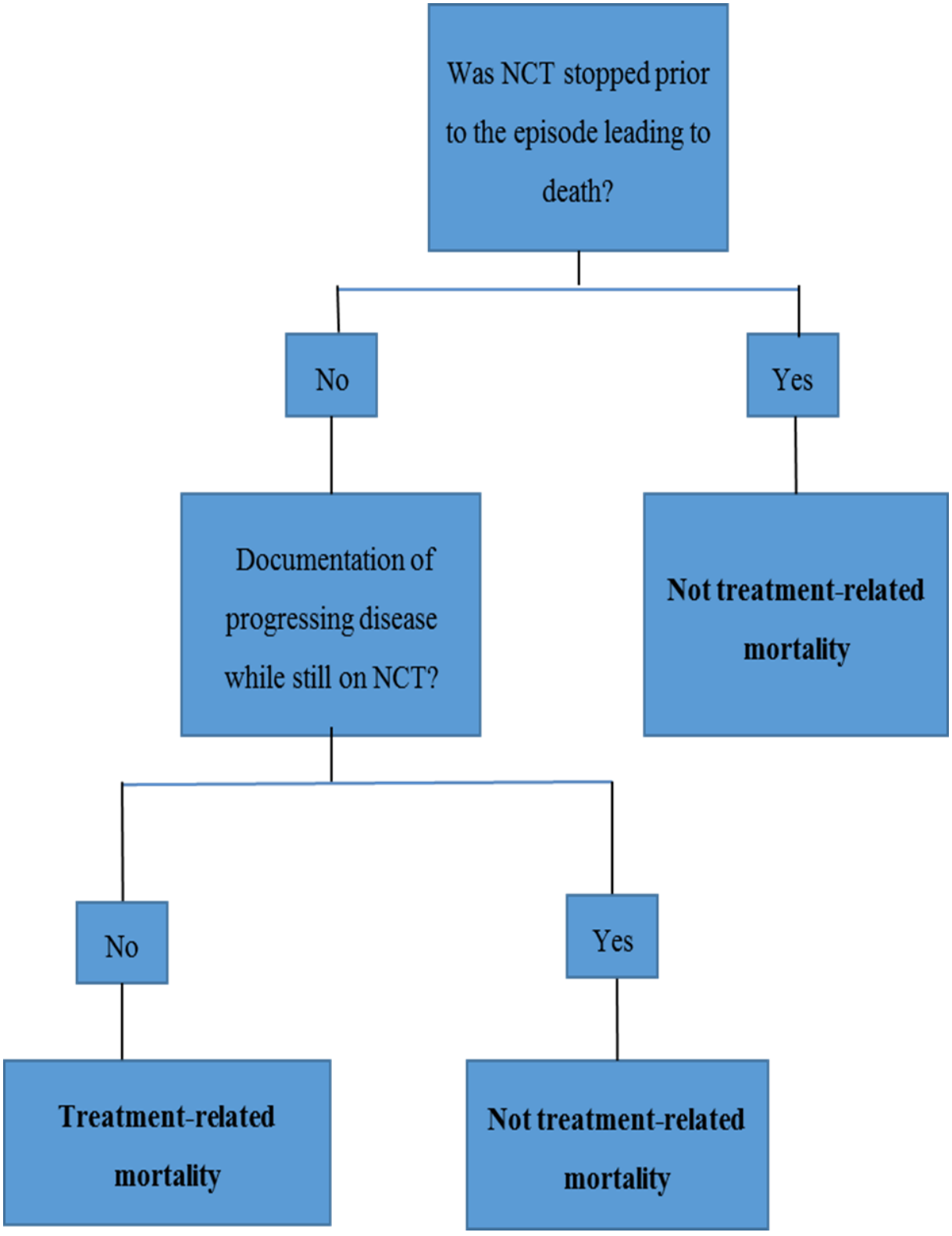
Conversely, it is also important to note how a diagnosis of cancer may be included in 'non-cancer deaths'. For example, a patient may commit suicide some years after the completion of treatment because of the psychological effects of their diagnosis or treatment. Any modification of the system would need to be sensitive to these potential issues.

We have further identified the need to refine the approach to categorising cause of death in cancer patients receiving care without intent to cure. This is particularly important if the system is applied in 'routine' settings, assessing deaths in the palliative setting rather than in the original setting of use within a curative trial. Increasingly, individuals destined not to be cured are living for lengthier periods due to participation in clinical trials/studies. This group of patients currently have all deaths classified as "not treatment-related mortality" as clinicians would have either specified progressive disease or that cancer therapy has no curative intent. This algorithm may fail to identify a significant group of patients who may die of causes amenable to better supportive care whilst receiving palliative care. For example, a patient can die of overwhelming sepsis whilst receiving palliative etoposide for refractory neuroblastoma. This could be addressed by modifying the algorithm for this type of use. Another proposal includes using a separate classification tool for patients on palliative care trials (figure 2), although this should be further developed in conjunction with palliative care physicians and researchers. The counter-argument to this suggested change is the risk of adding complexity to a simple, effective tool which can be used by people of different skills from different health care settings globally. It would also have similar issues with interpretation as with the original algorithm.

Reviewers failed to agree on a primary cause of death in 6 episodes and probable and possible causes in 4 cases. Differences in the cause of death allocated could be attributed to the reviewers' previous experience, clinical expertise and interpretation of the clinical records, particularly in light of potential previous direct clinical involvement with the cases under review. Currently, the tool is intended for use by any CRA. However, it may require users to have a certain level of experience or clinical expertise, and agreement may be reduced amongst CRAs who are new to the role.

Understanding and interpretation of the system as proposed for attribution of a specific mechanistic cause of death could potentially be improved by dedicating more time during the presentations and using the newly developed web-based training tool. Alternatively, the cause of death attribution system could be further refined.

Figure 2: Proposed classification of TRM in children receiving non-curative therapy (NCT) only.



2.6 Conclusions

This study has demonstrated the reproducibility and criterion validity of the TRM classification system. This supports the hypothesis that the classification system can be implemented quickly and effectively in different health care settings, thereby improving the consistency and accuracy of outcome reporting in clinical trials. The TRM classification system will be of immense value in the evaluation of deaths in the palliative setting. We propose the addition of a separate classification tool in patients on palliative trials.

Criterion validity was further established using the newly developed classification of TRM, demonstrating almost perfect agreement between CRAs and consultants k= 0.86 (95% CI 0.72-0.97) using a simple presentation and participation information leaflet. I believe the classification and cause of death attribution system could be implemented in different health care settings. This would help improve the consistency and accuracy of outcomes in clinical trials. Exploration for the use of the classification tool in patients receiving palliative intervention should be considered, with the possibility of refining the classification system for this group of patients.

Reviewers did not agree on 6 of the causes of death attributed to TRM. This may have been due to external factors, including previous experience and clinical knowledge. However, reliability can be improved by refining the training delivered to improve understanding and interpretation of what should be classified as the primary cause of death.

3 Systematic review and meta-analysis investigating the efficacy and safety of probiotics in people with cancer.

So far, this thesis has introduced key concepts regarding childhood cancers, including mortality, classification of death, and how supportive care strategies can be used to prevent and reduce morbidity and mortality.

The previous chapter reported on the validation of a classification system for treatment-related mortality for children diagnosed with cancer. Infection as a cause of death was identified as the most common cause of mortality from TRM: 6 out of the 10 patients who died from deaths due to TRM were attributed to infection.

Despite extensive research into the use of antibiotics, antiviral and antifungal supportive care strategies, infection-related mortality (IRM) is still a leading cause of TRM in children diagnosed with cancers.

Recent evidence has explored the relationship between mucositis and febrile neutropenia and this thesis has reported how currently there is no standard preventative or therapeutic intervention for febrile mucositis.

This, therefore led to the development of the next part of this thesis; exploring the use of probiotics as a novel strategy to reduce or prevent mucositis and infection in children with cancer.

This chapter has investigated the use of probiotics in gastrointestinal mucositis by reporting a systematic review and meta-analysis undertaken to investigate the efficacy and safety of probiotics.

3.1 Background

Probiotics are defined as “live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host” according to the World Health Organisation and United Nations Food and Agriculture Organization (FAO) (37). The most common strains belong to the genera *Lactococcus* and *Bifidobacterium* (38). Health benefits attributed to probiotics include the reduction of the severity of antibiotic-associated diarrhoea in paediatric patients (42), necrotising enterocolitis in premature infants (79) and the incidence of radiation-induced diarrhoea (80) (81).

Chemotherapy and radiotherapy-induced diarrhoea is a common adverse event. Radiotherapy is believed to potentially alter bacterial flora and affect the intestinal motility and vascular permeability of mucosal cells (44). Chemotherapy is thought to

alter the composition of intestinal flora and therefore affect the metabolism of intestinal enzymes vital for gut integrity. Changes to the gut flora may impact the gut defence barrier, immune function and absorption of vital nutrients (45). It is estimated that 20-45% of all chemotherapy patients experience severe diarrhoea (30). Radiotherapy or chemotherapy-induced diarrhoea may interrupt or even stop treatment, impair the quality of life and prolong hospital stay of patients with cancer, also potentially increasing health economic burdens (46).

There have been multiple studies investigating the role of probiotics in reducing chemotherapy and radiotherapy associated diarrhoea. A rigorous systematic review and meta-analysis investigating the efficacy and safety of use of probiotics in people with cancer by Redman et al were published in 2014 (47). It proposed that probiotics may reduce the severity and frequency of diarrhoea in patients with cancer following the review of 11 randomised-controlled trials (RCTs). A meta-analysis of 4 RCTs found that participants receiving probiotics, when compared to the control group, showed a significant reduction in the frequency of CTC grade ≥ 2diarrhoea (odds ratio (OR) 0.32, 95% confidence interval (CI) 0.13-0.79), and possibly grade ≥ 3 diarrhoea (OR 0.72, 95% CI 0.41-1.25). It suggested that in the probiotic groups soft/semi-solid stools may occur more commonly (OR 0.46, 95% CI 0.04-5.64) and reduce the need for anti-diarrhoeal medicine (OR 0.63, 95% CI 0.13-0.79). Between-study heterogeneity of results was noted. This was attributed to the different treatments, strain, doses and duration of probiotics alongside comorbidities, cancers and interventions delivered.

Safety analysis of 17 studies and 1530 cancer patients revealed 105 adverse events (AE) in 756 people consuming probiotics and 145 AE in 774 people not consuming probiotics. Adverse events included bacteraemia/fungaemias, infections, gastrointestinal symptoms, high blood pressure and raised intracranial pressure. Five case reports of the 756 cases describing the consumption of probiotics reported bacteraemia/fungaemia/blood culture growth. The use of probiotics in immunosuppressed patients is one of the most concerning adverse outcomes and requires further investigation. At the time of completion of the review by Redman et al (47) 10 trials were identified as ongoing, and few studies included children.

This review, therefore, aimed to update the systematic review and meta-analysis by Redman et al (47), to explore the previous heterogeneity and update the assessment of safety for the use for the use of probiotics in people with cancer.

3.2 An introduction to Systematic reviews

A systematic review is a methodological overview of primary research that focuses on a research question. Evidence is selected, synthesized and appraised according to a pre-specified eligibility criterion with the intention of minimising bias and heterogeneity by using precise, systematic methods. There are different ways of undertaking a systematic review, including the Cochrane method (82) or the Centre of Reviews and Dissemination (CRD) (81). A comprehensive systematic search of databases should take place following the identification of a question. The quality of the included studies should be assessed using an objective assessment tool to assess methodological quality as recommended by CRD (81) and the high-quality standards of the Cochrane collaboration (103). The review may include a narrative analysis or a meta-analysis; a statistical technique used to combine results across the included studies. Reporting and dissemination should be undertaken using methods recommended by the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) (115).

3.2.1 Why perform a systematic review?

Health care professionals are overwhelmed with large volumes of data that are difficult to manage. A systematic review is an efficient method to integrate and synthesize data across populations and subgroups to provide results for evidence-based decision making. Meta-analysis can be used to increase statistical power and precision of estimates of results from individual studies. Pre-defined eligibility criteria reduce bias, whilst an assessment of methodological quality identifies inherent biases in the primary studies and therefore improves the accuracy of conclusions drawn by the review.

3.2.2 Why perform this systematic review?

The systematic review was undertaken by Redman et al in 2014 (47) identified 10 ongoing clinical trials, and enough time had passed to undertake an update. A meta-analysis with more pooled studies would increase the statistical power and accuracy of point estimates and identify potential adverse complication. In addition to this, further subgroup analysis could be used to identify potential risk factors for complications/infection. The findings from this review were then used to identify areas of research which require more focus.

3.2.3 How to identify the evidence to be included in a systematic review

Undertaking a comprehensive search to identify relevant studies is necessary to minimise bias in the review process. The search strategy should be transparent and

recorded in a way that enables it to be replicated and evaluated to ascertain the same findings. A variety of sources can be searched to identify relevant data. These include:

- Searching electronic databases (e.g. PubMed and SCOPUS).
- Identifying key studies from reference lists from relevant studies.
- Searching relevant resources, key journals and conference proceedings.
- Directly contacting research authors and experts.
- Searching the references of included citations.

Undertaking narrow searching can introduce bias into the review process as it may exclude relevant data that is not in that source. For example, limiting searches to the use of electronic databases can introduce bias as they typically only identify published journal articles. This can result in publication bias as this type of database is unlikely to highlight studies that have not been published. Therefore, more comprehensive searches are necessary to limit the impact of publication bias. This may include searches for reports, abstracts or papers in other databases which includes conference reports or the search of grey literature.

Furthermore, limiting searches to certain languages can introduce language bias. Most journals in databases, such as MEDLINE and EMBASE, are only reported in English. Language bias can be overcome by including databases reporting journals from other languages. However, despite these issues, there are no agreed requirements for what an acceptable number of databases is to search.

3.2.4 Screening process

Screening of identified titles and abstracts commences once the search has been completed. Using one reviewer to undertake this poses a significant risk of selection and information bias. Having two reviewers independently screen titles/abstracts of identified studies to confirm inclusion and exclusion according to the inclusion criteria will reduce bias and increase the relevant number of studies identified for use in a systematic review. Disagreements between the independent reviewers can be resolved through mutual discussion or referred to a third independent reviewer.

3.2.5 Qualitative analysis

It is essential to undertake a critical appraisal of included studies by exploring the reporting and methodological qualities of a study. This is because the quality of a study can impact findings. Trials deficient in reporting and methodological quality can impair the accuracy of conclusions drawn due to high risk of bias. For example, if qualitative analysis demonstrated that two of three RCTs included in pooled statistical analysis

were of poor methodological quality (for example poor study design/implementation), it can infer uncertainty on any conclusions (i.e. statistical significance) drawn.

Using robust, validated tools specific for differing study designs ensures more objective, qualified assessment on findings. Examples of validated tools include The Cochrane Risk of Bias Assessment tool for RCTs (83), Risk of Bias in Non-randomised Studies of Interventions (ROBINS-I) for non-randomised studies (84) and the Loke method to assess the quality of studies investigating adverse effects (85).

3.2.6 Quantitative analysis

Systematic reviews may or may not include statistical analysis. These can be undertaken and reported in a variety of ways, such as findings reported from individual studies, pooled analysis from multiple studies (also known as a meta-analysis) or a pooled analysis of data from individual participants from multiple studies (also known as individual participant data). The systematic review protocol should report the strategy for data-synthesis a-priori to reduce reporting bias.

3.2.7 Pooled analyses

Pooled analysis of data increases the statistical power by increasing the total number of participants. This reduces random error, narrows confidence intervals (interval estimate that may include the true value for a population for a certain percentage, e.g. 95%) and precision intervals (the range of which the point estimate will fall in future studies for a certain percentage, e.g. 95%).

Meta-analyses are typically undertaken using two statistical models; fixed-effect and random-effect models. Fixed-effect models usually weight the results from each study according to the number of participants included and only factor variability of results reported between studies. Random-effect models adjust for between-study and within-in study variability. Whilst both approaches are similar, some argue using a fixed-effect model enables small studies to influence the estimate, whilst using a random-effect model models between-study variability, thereby reporting a more accurate statistic.

3.2.8 Heterogeneity

Studies included in systematic review and meta-analysis will have some variability in study outcomes, and this is known as heterogeneity. Types of heterogeneity include clinical heterogeneity (variability in participants, interventions and outcomes), methodological diversity (variability in study methodology and risk of evaluation) and statistical heterogeneity (variability of intervention effects being evaluated).

Variations between studies may result in differences in observed intervention effects because of random error or differences in studies. Heterogeneity can be explored by

visually inspecting a forest plot for the variability of effect reported between studies and poor overlap between confidence intervals suggests statistical heterogeneity.

Calculating chi-squared can suggest whether heterogeneity occurred because of chance. The I-sq statistic defines the percentage of heterogeneity that can be attributed to between-study difference rather than chance. I-sq values of 30-60%, 50-90% and greater than 75% suggest there is moderate, substantial and considerable heterogeneity respectively.

It is essential to identify statistical heterogeneity because reasons for variability between studies should be considered. Studies included in the pooled analysis with substantial differences can result in misleading meta-analysis results which can be overinflated. A subgroup meta-analysis using specific study characteristics can be undertaken to adjust for this.

3.3 Methodology

This review was undertaken followed a prespecified protocol registered on PROSPERO (the international register of systematic reviews): CRD 42016050252 October 2016 (13).

3.3.1 Aims

This review aimed to update the Redman et al (47) systematic review and meta-analysis safety analysis of the use of probiotics in people with cancer assessment.

It investigated the quality of identified randomised controlled trials and analysed quantitative outcomes, including the occurrence of invasive infection, duration of diarrhoea, and length of hospital stay from identified studies.

The review also investigated the safety of using probiotics in patients with cancer by investigating reported adverse events.

3.3.2 Inclusion criteria

Designs of studies eligible for efficacy analysis included randomised-controlled trials of people diagnosed with cancer who received probiotics as an intervention. Outcomes assessed included antibiotic-associated diarrhoea, gastrointestinal infection, mucositis or any adverse event. Non-randomised studies and case reports were also included within the safety analysis.

3.3.3 Identification of trials

Database searches of MEDLINE, EMBASE, and Allied and Complementary Medicine (AMED) without language limitations were undertaken with the following search strategy:

((cancer OR malignancy OR malignant OR oncology OR oncological OR transplant OR leukaemia tumour OR tumour OR chemotherapy OR radiotherapy) AND (probiotic OR lactobacillus OR saccharomyces)) AND (infection OR sepsis OR diarrhoea OR fungal))

A simplified search strategy was used for the following search engines: the Cochrane Central Register of Controlled Trials, Literatura Latino-Americana e do Caribe em Ciências da Saúde, Database of Abstracts of Reviews of Effects, American Society of Clinical Oncology, International Society of Paediatric Oncology, Multinational Association of Supportive Care in Cancer, International Cancer Research Portfolio, National Cancer Institute Clinical Trials, National Cancer Research Institute, Current Controlled Trials and CenterWatch.

3.3.4 Study selection

Study selection and data extraction were conducted in 2 stages:

- Two reviewers independently assessed the title and abstract of the studies for possible inclusion (H.H, M.R). Inclusion or exclusion was verified by assessing the full text of potentially included studies.

Discrepancies between the raters were addressed, and those unresolved were referred to an independent assessor (R.P).

- Data was extracted by a researcher using a standardised form (H.H) which was independently checked by a second person (M.R). When further information was required, the author of the paper was contacted.

The study selection process and data extraction were piloted using a sample of 100 papers in order to check that the correct papers would be identified, interpreted and analysed. The pilot study was used to refine the inclusion criteria to ensure it could be applied consistently, and that correct data were extracted.

3.3.5 Risk of bias and quality assessment of included studies

The Cochrane risk of bias tool was used to assess the risk of bias of included RCTs (83).

The Loke method was used to assess the quality of studies investigating adverse effects (85). Items were identified as “unclear risk of bias” when studies did not specify the relevant information.

3.3.6 Data synthesis

Where possible, comparable data were pooled using the Mantel-Haenszel method for dichotomous data and inverse variance model for continuous data as recommended in “Systematic reviews: CRD's guidance for undertaking reviews in health care” (86). This was undertaken using random-effect meta-analyses to supply an average estimate of effects, with their associated 95% confidence interval (CI) and 95% prediction interval (PI) (87). Results were displayed in forest plots. I-sq was used to evaluate between-study heterogeneity. An I-sq of >50% was deemed to represent significant heterogeneity (86). Funnel plots were planned to be used to assess for bias. However, there were insufficient data to undertake this. The analysis was undertaken using the ‘metafor’ package in R-studio (74) .

3.3.6.1 Subgroup analysis

It was not possible to undertake any subgroup analysis due to marked heterogeneity of included studies. Subgroup analyses were intended to assess age of patients, type of probiotics, mode of delivery, and underlying cancer therapy based on radiotherapy or chemotherapy interventions.

3.4 Results

We identified 8015 unique articles, of which 98 were selected for full-text review, with 10 RCT and 8 additional safety papers added in this update (see Figure 3). This resulted in a total of 21 studies included in the efficacy analysis and 25 studies in the safety analysis. Indications for excluding articles following full-text review are summarised in Figure 3.

3.4.1 Efficacy analysis

Table 7 summarises characteristics of the RCTs included in the efficacy analysis. Studies were conducted in 14 different countries, of which China was the most common. Eleven studies included surgical interventions, 9 studies included radiotherapy, and 7 studies included chemotherapy interventions. Sixteen studies used probiotics with more than one strain of bacteria, and 11 studies included 3 or more strains of bacteria. Eighteen studies included *Lactobacillus* strains, of which there were 12 different species. Fifteen studies included *Bifidobacterium* strains, of which there were 7 different species. Only 2 of the 21 studies included paediatric patients.

3.4.2 Risk of bias assessment

Findings of the risk of bias assessment identified that most items were assessed as unclear (up to 45% in each domain) due to a lack of reporting of methods in both the reports and published protocols (table 8). The highest risk of bias was noted when assessing performance bias (29%). Most of the studies reported as high risk specified that participants but not personnel were blinded to the intervention delivered, which could potentially have affected how outcomes were assessed. Lowest risk of bias was found when investigating attrition bias and sequence generation (62% and 52% respectively); most studies clearly specified methods used. Risk of bias assessments are summarised in table 8 and figure 4.

3.4.3 Meta-analysis

Pooled analysis demonstrated that probiotics reduced the incidence of diarrhoea in patients with cancer [odds ratio (OR) = 0.52, 95% CI 0.34-0.78, 95% PI 0.30-0.92, I-sq 36.9%, 5 studies, figure 5], and duration of pyrexia [standardized mean difference 0.64 days, 95% CI 053-0.77, I-sq 0.01%, 5 studies, figure 6]. Probiotics may also reduce the severity of diarrhoea, for example Common Toxicity Criteria grade 2 diarrhoea [OR=0.67, 95% CI 0.15-2.98, PI 0.07-6.55, I-sq 76.9%, 3 studies], grade 3 and 4 diarrhoea [OR=0.51, 95% CI 0.12-2.2, PI 0.03-9.08, I-sq 92.5%, 4 studies, figure 7], the incidence of septicaemia [OR=0.39, 95% CI 0.13-1.17, PI 0.05-3.05, I-sq 76.4%, 5 studies], and central line infections [OR=0.50, 95% CI 0.15-1.71, PI 0.09-2.7, I-sq 62.9%, 3 studies] but these results are very heterogenous and uncertain. Due to the marked heterogeneity of reporting in included studies we were unable to perform subgroup analysis on intervention, strain, dose of probiotic and age.

3.4.4 Safety of probiotics

Demographics of the 25 studies (N = 2,242) included in the safety analysis are summarised in Table 9 at the end of this chapter. An estimated 237 AEs events occurred in those consuming probiotics and 314 AEs in those not consuming probiotics. However, most studies did not specify how AEs were reported; for example, it is unclear whether two separate AEs recorded as ‘sepsis’ or ‘pneumonia’ occurred independently or from the same episode. No deaths attributed to probiotics were identified in the update. In the initial review, 2 deaths were reported in probiotic groups, but these were not attributed to the intervention delivered. Five case reports were identified during the initial review of probiotic associated infections, and no further case reports or probiotic associated infections were identified in the update, with one cohort study explicitly reporting an absence of probiotic associated infection.

3.4.5 Loke method for quality assessment for the reporting of adverse events

Quality assessments of studies included for safety analysis are reported in Table 10 at the end of this chapter. As described in the initial review definitions of adverse events were inconsistently reported. Some were defined according to CTCAE or NCI-CTC, whereas others did not state how the definition was determined.

3.5 Discussion

3.5.1 Summary of main results

This update found 10 new RCTs and 8 further studies reporting AEs of probiotics in people with cancer, giving a total of 21 studies for efficacy analysis and 25 studies for safety analysis. There was marked heterogeneity of the strain, dose, and duration of probiotic used and age, cancers and anti-cancer therapies under study. It was not possible to undertake subgroup analysis to explore between-study heterogeneity further.

3.5.2 Strengths of this systematic review and meta-analysis

This review was performed in accordance with standards published by the Centre of Reviews and Dissemination(81). A comprehensive search strategy of 14 databases was undertaken, and this included a grey literature search. We created general inclusion criteria to identify different study types. Two reviewers performed abstract screening independently and cross-checked decisions to minimise bias. Although the data extraction was completed by one person, data from included papers and full-text exclusions was verified by a second reviewer. The Cochrane Risk of Bias was used for the randomised-controlled trials to assess biases as a measure of intervention effect, and the Loke method was used to assess the quality of studies investigating adverse effects. Where possible meta-analysis was undertaken to calculate pooled effects to strengthen findings further.

3.5.3 Comparisons with other reviews

The systematic review undertaken by Redman et al (47) reported that there was insufficient evidence to claim probiotics are effective and safe. Meta-analyses undertaken demonstrated that probiotics significantly reduce the incidence of CTC grade > 2 diarrhoea and may reduce the incidence of CTC grade > 3 diarrhoea, daily bowel movements, and the need for anti-diarrhoeal medication. The review suggested that an effect on faecal bacteriological composition may be found, but this needs to be examined in further trials alongside analysis of ongoing studies before drawing any conclusions.

The updated systematic review undertaken for this thesis concluded there is still insufficient evidence to determine that probiotics are effective and safe in people with cancer. A meta-analysis demonstrated that probiotics might reduce the incidence of diarrhoea, duration of pyrexia and may reduce incidence of septicaemia and central line infection. However, these results should be interpreted cautiously because of the heterogeneous nature of included studies and the lack of studies with a clear low risk of

bias. It was not possible to perform subgroup analysis, particularly in children, to investigate this further. Our review reported that probiotics may be a rare source of infection but that no deaths have been attributed to their consumption. However, the variability of definitions used and reporting of adverse events means conclusions cannot be drawn with confidence. There was still insufficient evidence to investigate the effect on faecal bacteriology, highlighting the need for further research.

A further systematic review (88) was identified during the screening process but was not eligible for inclusion. It reported that patients receiving chemotherapy or radiation therapy demonstrated changes in intestinal microbiota, particularly, a decrease in *Bifidobacterium*, *Clostridium* species, *Faecalibacterium prausnitzii*, and increase in *Enterobacteriaceae* and *Bacteroides,* which may increase the risk of developing mucositis, and that probiotics significantly reduce the incidence of diarrhoea. However, these conclusions were drawn from studies which included pre-clinical models, and the methodology for how the systematic review was undertaken was not reported. Whilst our systematic review did not identify sufficient data to undertake a meta-analysis of the faecal composition of stool samples in clinical trials, and it has identified the need for further trials to explore this further.

3.5.4 Limitations of this systematic review and meta-analysis

3.5.4.1 Risk of bias assessment of trials

Domains of risk of bias were mostly reported as unclear due to limited reporting of methods undertaken. The highest risk was identified when assessing selection and detection bias. Whilst aspects of these biases may not be relevant, e.g. whether participants were blinded to the episodes of diarrhoea, most studies did not report sufficient information about methods undertaken, e.g. whether personnel were blinded from allocation of randomisation. This may have undermined the randomisation process, resulting in biased and inflated effect estimates. Selection bias can be reduced by implementing allocation concealment. Accuracy of the assessment of bias could be improved by more transparent reporting in studies and protocols. Reporting of studies can be improved by using the Consolidated Standards of Reporting Trials (CONSORT)(89). This is an evidence-based set of recommendations for the reporting of RCTs enabling better transparency and appraisal of studies, thereby potentially reducing bias.

3.5.4.2 Quality assessment for the reporting of adverse events

The Loke method (85) for the quality assessment of safety of probiotics (Table 10) identified that studies are still unclear on definitions, measure, and reporting of adverse

events. Definitions of adverse events may vary according to country and health care provisions.

Currently, no consistent definitions are used in the reporting of adverse events and other outcomes. Uniformity of outcome reporting can be improved using the Core Outcome Measures in Effectiveness Trials (COMET) initiative (90) in which a standardised set of outcomes (i.e. adverse events) represent the minimum measured and reported in clinical trials. We were unable to perform subgroup analysis due to the number of studies using different strains and doses of probiotics, age groups, treatment, and reporting of different outcomes. Using the proposed COMET initiative to agree on a standardised set of outcomes would enable improved accuracy when undertaking further updates, potentially reducing between-study heterogeneity.

3.5.4.3 Efficacy of probiotics

There remain insufficient studies to assess the true effect of probiotics in people with cancer. Meta-analysis suggests probiotics may be beneficial, but further studies are still required, particularly in children. The updated meta-analysis was unclear if probiotics can reduce the severity of grade 2 diarrhoea [OR=0.67, 95% CI 0.15-2.98, PI 0.07-6.55, I-sq 76.9%, 3 studies] or Grade 3 and 4 diarrhoea [OR=0.51, 95% CI 0.12-2.2, PI 0.03-9.08, I-sq 92.5%, 4 studies]. Pooled analysis did demonstrate that those treated in the probiotic group had a reduced incidence of diarrhoea [odds ratio (OR) = 0.52, 95% confidence interval (CI) 0.34-0.78, PI 0.3-0.92, I-sq 36.9%, 5 studies] and reduced duration of pyrexia [standardized mean difference 0.64 days, 95% CI 0.53-0.77, 5 studies]. It was unclear if probiotics can reduce the incidence of septicaemia [OR=0.39, 95% CI 0.13-1.17, PI 0.05-3.05, I-sq 76.4%, 5 studies] or central line infections [OR=0.5, 95% CI 0.15-1.71, PI 0.09-2.7, I-sq 62.9%, 3 studies].

Marked heterogeneity was demonstrated by the high I-sq results and wide prediction intervals. Prediction intervals represent an estimate of where the effect will fall in future observations. Wide prediction interval, therefore, demonstrates a greater variability of estimated treatment effects in future studies. This could be attributed to clinical diversity, e.g. the use of different strains and doses of probiotics, cancer diagnoses and interventions delivered, methodological diversity, e.g. differing study designs and statistical heterogeneity, e.g. the varying outcome effects reported in studies.

There were insufficient data reported in the studies identified to undertake an updated pooled analysis of daily bowel movements, use of anti-diarrhoeal medication, and faecal bacteriological comparison. It was not possible to undertake any subgroup analysis due to the marked variability of study designs, probiotic strain dose, age and outcomes reported, and the small numbers of studies in each subgroup. Again, using

the COMET initiative to create a standardised set of outcomes would enable more accurate meta-analysis and therefore potentially more accurate conclusions.

3.5.4.4 Safety analysis

Twenty-five studies (N = 2,242) were included in the updated safety analysis summarised in table 10 at the end of this chapter. It is unclear how many individuals sustained adverse events as reporting varied between studies. Some studies reported on individual events rather than people sustaining an adverse event, and it is unclear how this may overlap (for example some studies reported on the incidence of septicaemia, incidence of pneumonia and UTIs - making it challenging to identify the number of individuals, or indeed if the same episode of illness was counted in two categories). An estimated 237 AEs events occurred in those consuming probiotics and 314 AEs in those not consuming probiotics. Of the 8 studies identified during the updates, there were no deaths attributed to probiotics. In the initial review, 2 deaths were reported in probiotic groups, but this was not attributed to the intervention. There were 5 case reports identified during the initial review of associated probiotic infections. Some studies did not report on bacterial isolates from positive blood cultures identified (in both probiotic and control groups). Therefore, it cannot be concluded with confidence that there were no probiotic-associated infections, or that adverse events sustained cannot be attributed to probiotics consumed, due to the heterogeneity of malignancies and treatment regimens. As adverse events were also not clearly or uniformly defined in identified studies, it cannot be determined if all relevant data were appropriately identified, recorded or documented. As previously explained, this could be improved using methods such as the COMET initiative in future studies.

3.6 Conclusion

This systematic review update demonstrates that there is still insufficient evidence to conclude that probiotics are effective and safe in people with cancer. Meta-analysis has demonstrated that probiotics may reduce the incidence of diarrhoea, duration of pyrexia and may possibly incidence of septicaemia and central line infection. However, these results should be interpreted cautiously because of the heterogeneous nature of included studies and the lack of studies with a clear low . It was not possible to perform subgroup analysis, particularly in children, to investigate this further. Probiotics may be a rare source of infection, but no deaths have been attributed to their consumption. However, the variability of definitions used and reporting of adverse events means conclusions cannot be drawn with confidence. Further harmonisation of reporting of clinical trials using strategies such as the COMET initiative and CONSORT checklist would enable greater precision and confidence in conclusions drawn.

Figure 3: Summary of the screening process.

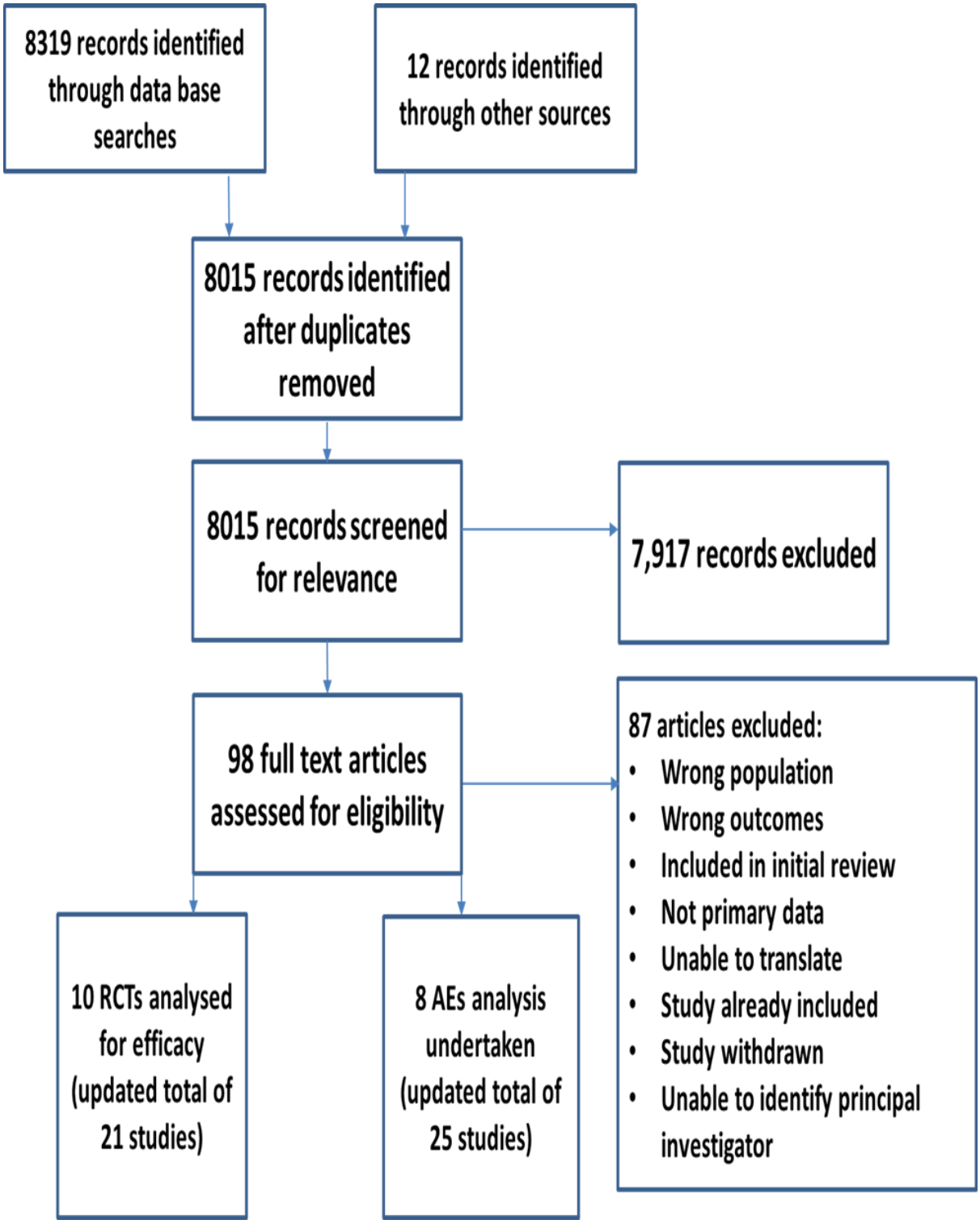


Table 7: **Characteristics of included RCTs for efficacy analysis**

Bold: studies identified during update, RT, Radiotherapy; CHT, chemotherapy.

Study first author	Country of study	Study	Probiotic administered
Sadahiro	**Japan**	**Surgery**	***Bifidobacteria.***
Delia	**Italy**	**Surgery, RT**	**VSL#3** **Four strains of *Lactobacilli* (*L. casei, L. plantarum, L. acidophilus,* and *L. delbruekii subsp. bulgaricus*),** **Three strains of *Bifidobacteria* (*B. longum, B. breve*, and *B. infantis*),** **One strain of *Streptococcus salivarius subsp. thermophilus*.**
Demers	**Canada**	**RT**	**Double strain Bifilact® probiotics (*Lactobacillus acidophilus* LAC-361 and Bifidobacterium longum BB-536)**
Ekert	**Austrailia**	**CHT**	**Co-trimoxazole and synerlac (*Lactobacilli* preparation)**
Kotzampassi	**Greece**	**Surgery**	**Four probiotics: *Lactobacillus acidophilus* LA-5, *Lactobacillus plantarum, Bifidobacterium lactis* and *Saccharomyces boulardii***
Liu ZH	**China**	**Surgery**	***Lactobacillus plantarum*, *Lactobacillus acidophilus*-11 and *Bifidobacterium longum*-88**

Study first author	Country of study	Study	Probiotic administered
Liu Z	**China**	**Surgery**	**Three PRO bacteria composed of *Lactobacillus plantarum* (CGMCC No.1258), *Lactobacillus acidophilus* and *Bifidobacterium longum* every day**
Mego	**Slovakia**	**CHT**	**Each capsule contained 10 lyophilized probiotic strains including *Bifidobacterium breve* HA-129 (25%), *Bifidobacterium bifidum* HA-132 HA (20%), *Bifidobacterium longum* HA-135 (14.5%), *Lactobacillus rhamnosus* HA-111 (8%), *Lactobacillus acidophilus* HA-122 (8%), *Lactobacillus casei* HA-108 (8%), *Lactobacillus plantarum* HA-119 (8%), *Streptococcus thermopilus* HA-110 (6%), *Lactobacillus brevis* HA-112 (2%), *Bifidobacterium infantis* HA-116 (0.5%)**
Yang	**China**	**Surgery**	***Bifidobacterium longum*, *Lactobacillus acidophilus* and *Enterococus faecalis***
Zhang	**China**	**Surgery**	***B longum, L acidophilus and Enterococcus faecalis***
Castro [14]	Brazil	RT	*Lactobacillus casei shirota* and *Bifidobacterium breve*
Chitapanarux [22]	Thailand	RT	*Lactobacillus acidophilus, Bifidobacterium bifidum* (Infloran®)

Study first author	Country of study	Study	Probiotic administered
Delia [23]	Italy	RT	VSL#3 (*Lactobacillus casei, Lactobacillus plantarum, Lactobacillus acidophilus, Lactobacillus delbrueckii subsp. Bulgaricus, Bidobacterium longum, Bifidobacterium breve, Bifidobacterium infantis, Streptococcus salivarius subsp. thermophilus*)
Germain [13]	Canada	RT ± CHT ± surgery	*Bifilact (Lactobacillus acidophilus LAC-361 and Bifidobacterium longumBB-536)*
Gianotti [24]	Italy	Surgery	*Lactobacillus johnsonii, Bifidobacterium longum (with maltodextrin)*
Giralt [12]	Spain	RT ± CHT	*Lactobacillus casei DN-114 001, Streptococcus thermophilus, Lactobacillus delbrueckii subsp. Bulgaricus*
Liu [25]	China	Surgery	*Lactobacillus plantarum, Lactobacillus acidophilus, Bifidobacterium longum*
Osterlund [26]	Finland	Adjuvant CHT following surgery	*Lactobacillus rhamnosus*
Sharma [27]	India	RT + CHT	*Lactobacillus brevis*
Urbancsek [28]	Hungary	RT	*Lactobacillus rhamnosus*
Wada [29]	Japan	CHT	*Bifidobacterium breve strain Yakult (BBG-01)*

Table 8: Risk of bias for included randomised controlled trial for efficacy analysis, judged according to Cochrane risk of bias assessment tool (82)

Bold=studies identified in updated search

Study first author	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias): all outcomes	Selective reporting (reporting bias)
Sadahiro	Low risk	Low risk	Unclear	Low risk	Low risk	Unclear
Delia	Higher risk	Unclear	Unclear	Unclear	Unclear	Unclear
Demers	Higher risk	Higher risk	Low risk	Low risk	Low risk	Low risk
Ekert,	Higher risk	Higher risk	Unclear	Unclear	Unclear	Unclear
Kotzampassi	Lower risk	Low risk	Unclear	Low risk	Low risk	Low risk
Liu ZH	Lower risk	Unclear	Higher risk	Higher risk	Low risk	Unclear
Liu Z	Unclear	Low risk	Higher risk	Unclear	Low risk	Unclear
Mego	Low risk	Low risk	Low risk	Unclear	Unclear	Higher risk
Yang	Low risk	Low risk	Low risk	Unclear	Low risk	Unclear
Zhang	Unclear	Unclear	Higher risk	Unclear	Unclear	Unclear
Castro [14]	Low risk	Low risk	Low risk	Unclear	Higher risk	Unclear

Study first author	Random sequence generation (selection bias)	Allocation concealment (selection bias)	Blinding of participants and personnel (performance bias)	Blinding of outcome assessment (detection bias)	Incomplete outcome data (attrition bias): all outcomes	Selective reporting (reporting bias)
Chitapanarux [22]	Unclear	Unclear	Low risk	Low risk	Low risk	Low risk
Delia [23]	Unclear	Unclear	Low risk	Unclear	Low risk	Unclear
Germain [13]	Low risk	Higher risk	Low risk	Low risk	Unclear	Unclear
Gianotti [24]	Low risk	Low risk	Low risk	Low risk	Unclear	Unclear
Giralt [12]	Unclear	Unclear	Low risk	Low risk	Low risk	Low risk
Liu [25]	Low risk	Unclear	Higher risk	Unclear	Low risk	Unclear
Osterlund [26]	Low risk	Unclear	Higher risk	Higher risk	Low risk	Unclear
Sharma [27]	Low risk	Low risk	Unclear	Unclear	Unclear	Unclear
Urbancsek [28]	Unclear	Unclear	Unclear	Higher risk	Low risk	Higher risk
Wada [29]	Higher risk	Higher risk	Higher risk	Higher risk	Low risk	Low risk

Figure 4: Bar chart demonstrating of Risk of Bias results

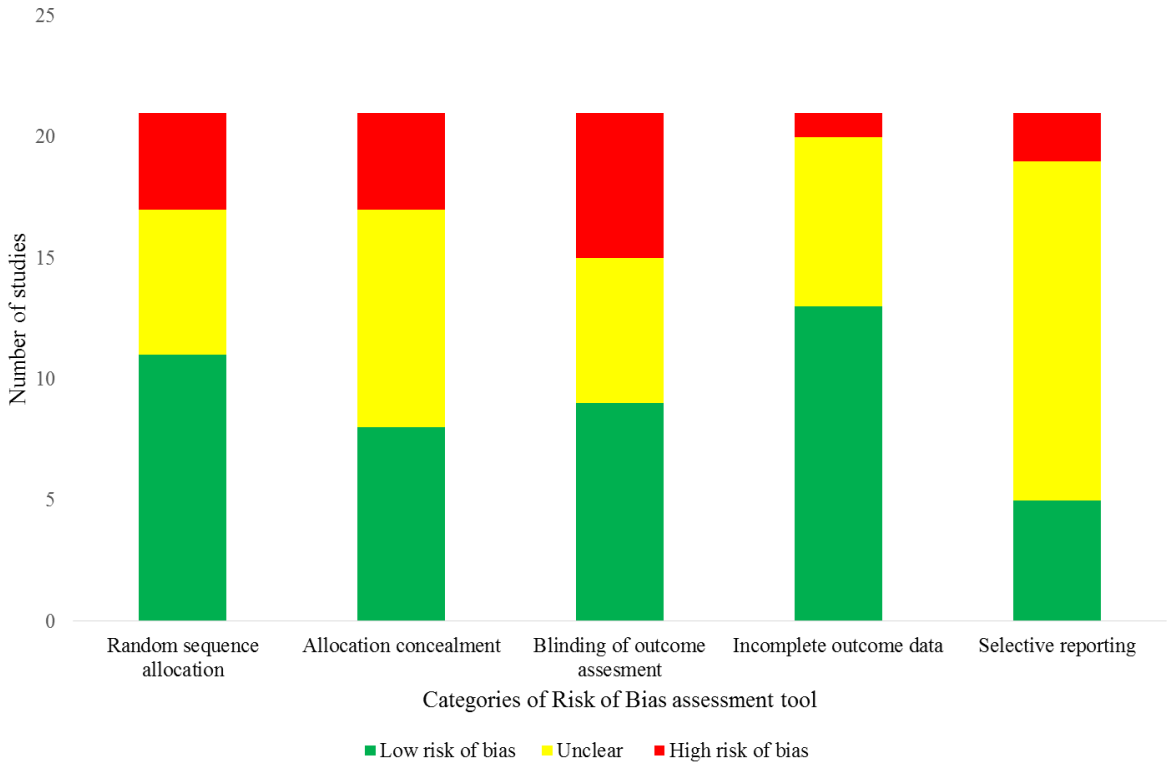
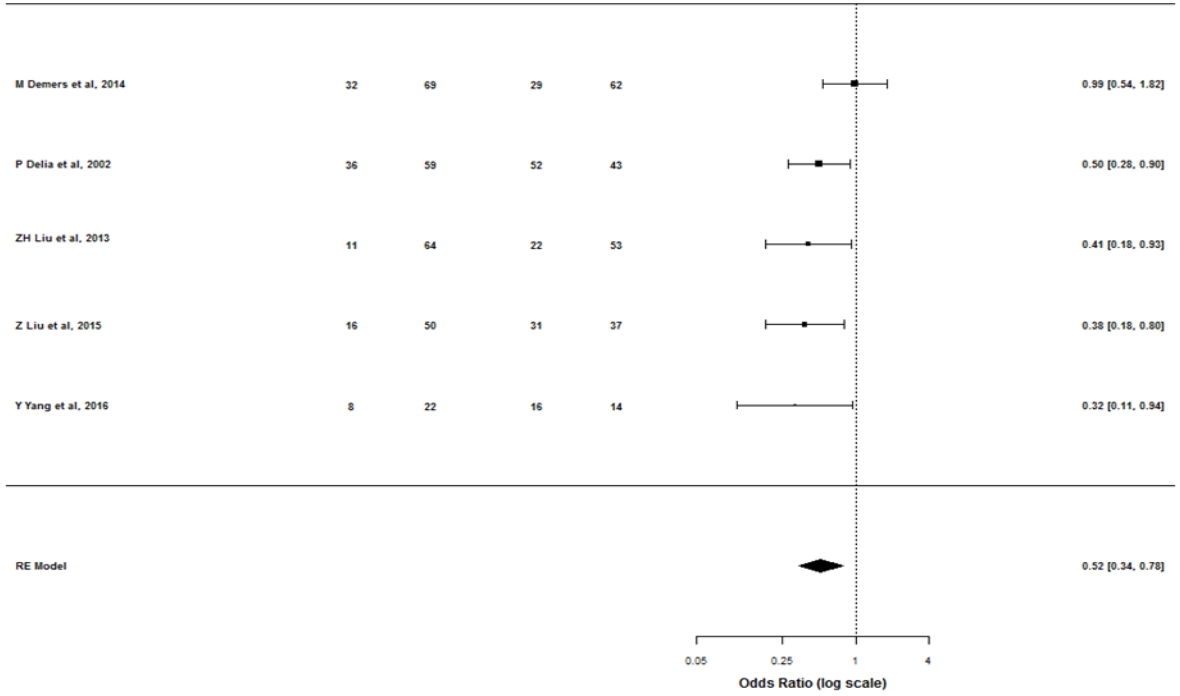


Figure 5: Forest plot summarising for the incidence of diarrhoea



Favours probiotic **Favours control**

Figure 6: Forest plot summarising the duration of pyrexia (days)

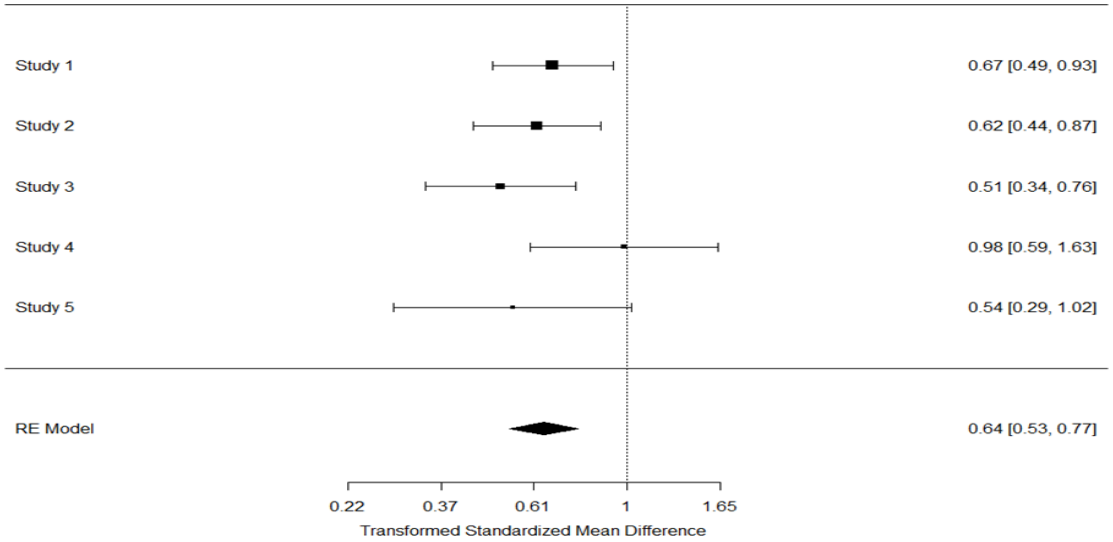
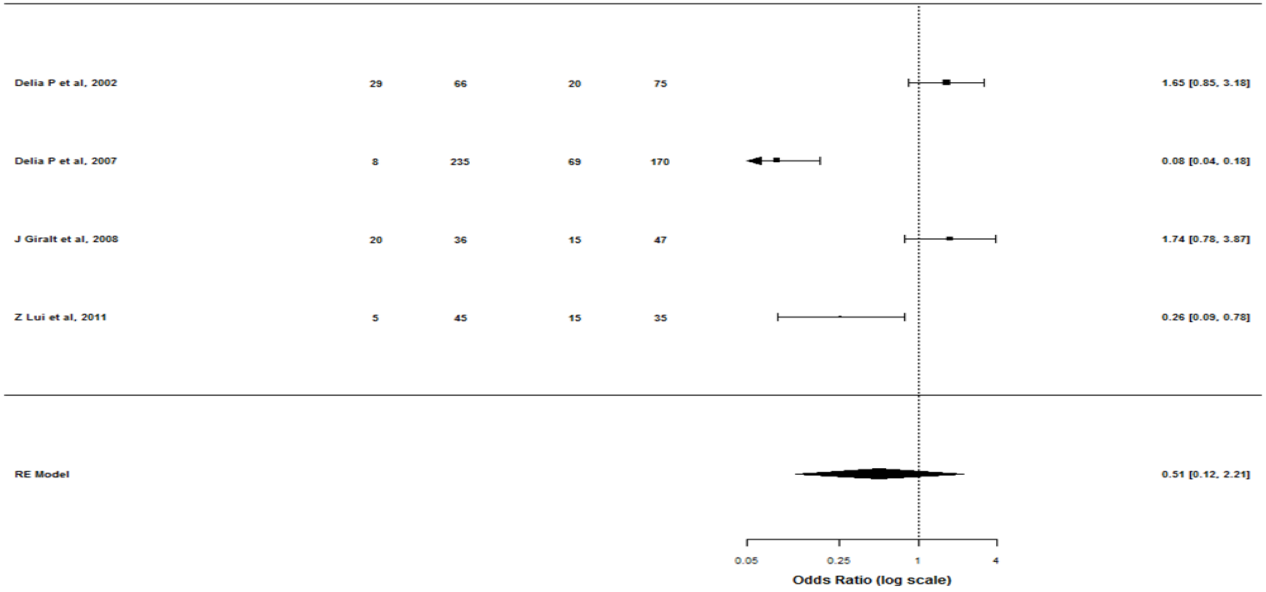


Figure 7: Forest plot summarising grade >3 and 4 diarrhoea



Favours probiotic **Favours** **control**

Table 9: Studies included for safety analysis

Bold= studies identified during update

Study first author	Study design		Probiotic administered	Adverse event
Ekert,	**RCT**	**Total 68** **Intervention: 35** **Fracon: 33** **Control: 34**	**Co-trimoxazole and Synerlac (*Lactobacilli* preparation).**	**Intervention group (Co-trimoxazole and Synerlac)** **5 x fevers >38 C appearing for the first time, 2 positive blood cultures,5 x fevers >38 C appearing for the first time, 2 positive blood cultures.** **No issues regarding tolerance to treatment observed** **FRACON group:** **5 x fevers >38 C appearing for the first time, 5 positive blood cultures, 19 (? episodes) vomiting & nausea, 7 refusal to take medication, 9 dose reductions, 5 changed to alternate regimens** **Control group:** **14 fevers >38 appearing for the first time, 8 positive blood cultures.**

Kotzampassi	**RCT**	**Total 164** **Probiotic: 80** **Control:80**	**Four probiotics: *Lactobacillus acidophilus* LA-5, *Lactobacillus plantarum, Bifidobacterium lactis* and *Saccharomyces boulardii*.**	**Probiotic group:** **24 (28.6%) major complications, 10 (11.9%) any infectious complications,** **2 (2.4%) pneumonia, 6 (7.1%) surgical site infections, 4 (4.8%) bacteraemias** **6 (7.1%), severe sepsis, 1(1.2%) anastomosis leakage,1(1.2%) need for mechanical ventilation** **Isolates- *Acinetobacter* 3 (3.7 %), *Pseudomonas aeruginosa* 2 (2.3 %), *methicillin-resistant Staphylococcus aureus* 1 (1.2 %).** **Control group:** **39 (48.8%) major complications, 23 (28.7%) any infectious complications, 9 (11.3%) pneumonia, 16 (20%) surgical site infections, 8 (10%) bacteraemia,** **8 (10%) severe sepsis, 4 (5%) anastomosis leakage, 7 (8.8%) and need for mechanical ventilation, 1 patient sustained a pulmonary embolism** **Isolates- *Acinetobacter* 8 (10.0 %), *Pseudomonas aeruginosa* 4 (5.0 %), *methicillin-resistant Staphylococcus aureus* (MRSA) 3 (3.8 %).**
Liu ZH	**RCT**	***Total: 150*** ***Probiotic: 75*** ***Control:75***	***Lactobacillus plantarum*, Lactobacillus *acidophilus*-11 and *Bifidobacterium longum*-88**	**Probiotic group:** **41 (55%) septicaemia, 4 (5%) central line infection, 3(4%) pneumonia, 2 (3%) UTI, 11 (15%) incidence of diarrhoea** **5.82 days+/-1.98 SD duration of post-operative**

				pyrexia ***Escherichia coli*: Blood 3, Central lines 1, sputum 1** ***Staphylococcus aureus*: Blood 1, Central lines 1, sputum 2** ***Klebsiella pneumoniae*: Blood 0, Central lines 0, sputum 1** ***Aeruginosum*: Blood 0, Central lines 1, sputum 1** **Control group:** **41 (73%) septicaemia, 12 (16%) central line infection, pneumonia 10 (13%),** **UTI 10 (13%), 29 (22%) incidence of diarrhoea,** **6.68 days+/-2.29 SD duration of post-operative pyrexia** **Escherichia coli: Blood 7, Central lines 3, sputum 3** **Staphylococcus aureus: Blood 3, Central lines 3, sputum 2** **Klebsiella pneumoniae: Blood 0, Central lines 0, sputum 0** ***Aeruginosum*: Blood 0, Central lines 2, sputum 0**
Liu Z	**RCT**	**Total: 150** **Probiotic: 66** **Control: 68**	**Three PRO bacteria composed of LP (CGMCC No.1258), LA-11 and BL-every day**	**Probiotic group:** **39 (59%) septicaemia, 7 (11%) central line infection, 6 (9 %) pneumonia** **2 (3%) UTI, 16 (24%) incidence of diarrhoea**

				35 (51%) abdominal distention, abdominal cramping 15 (23%) **6.02 days+/-1.68 SD duration of post-operative pyrexia** ***Escherichia coli*: Blood13, Central lines 0, sputum 1** **Staphylococcus aureus: Blood 1, Central lines 1, sputum 1** **Klebsiella pneumoniae: Blood 0, Central lines 0, sputum 1** ***Aeruginosum*: Blood 0, Central lines 1, sputum 0** **Control group:** **60 (88%) septicaemia, 6 (9%) central line infection, 8 (12%) pneumonia** **9 (13%) UTI, 31 (46%) incidence of diarrhoea, 6.98 days+/-2.22 SD duration of post-operative pyrexia** ***Escherichia coli*: Blood 6, Central lines 1, sputum 2** ***Staphylococcus aureus*: Blood 2, Central lines 1, sputum 2** ***Klebsiella pneumoniae*: Blood 0, Central lines 0, sputum 1** ***Aeruginosin*: Blood 1, Central lines 0, sputum 0**
Mego	**RCT**	**Total: 46** **Probiotic: 23**	**Each capsule contained 10 lyophilized probiotic strains including *Bifidobacterium***	**Probiotic group:** **Diarrhoea grade 1: 5 (21.7%), diarrhoea grade 2:4 (17.4%), diarrhoea grade 3: 0, diarrhoea**

		Control: 23	***breve* HA-129 (25%), *Bifidobacterium bifidum* HA-132 HA (20%), *Bifidobacterium longum* HA-135 (14.5%), *Lactobacillus rhamnosus* HA-111 (8%), *Lactobacillus acidophilus* HA-122 (8%), *Lactobacillus casei* HA-108 (8%), *Lactobacillus plantarum* HA-119 (8%), *Streptococcus thermopilus* HA-110 (6%), *Lactobacillus brevis* HA-112 (2%), *Bifidobacterium infantis* HA-116 (0.5%).**	**grade 4: 0, enterocolitis: bloating: 2 (8.7%)** **Control group:** **Diarrhoea grade 1: 8 (34.8%), diarrhoea grade 2: 2 (8.7%), diarrhoea grade 3: 0 (13%), diarrhoea grade 4: 1 (4.3%) enterocolitis: 2 (8.7%), bloating: 4 (17.4 %)**
Mego	**Cohort**	***N=60***	**E. faecium M-74**	**14 patients (100%) with infectious fever** **30 infectious episodes** **14 (47%) microbiologically documented infection (episodes)** **10 (33%) fever of unknown origin** **Blood stream: 5 *Coagulase-negative staphylococcus*, 1 *Escherichia coli*, 1 *Klebsiella pneumonia*, 2 *Corynebacterium sp*, 1 *Pseudomonas aeruginosa*, 1 *Stenotrophomonas maltophilia.* 1 *Citrobacter sp.*** **Urinary tract: 1 *Enterococcus faecalis*, 1 *Escherichia coli.*** **No Probiotic associated infections or**

				bacteraemia.
Yang	**RCT**	**Total: 60** **Probiotic: 30** **Control: 30**	***Bifidobacterium longum*, *Lactobacillus acidophilus* and *Enterococus faecalis*.**	**Probiotic:** **4.77 days +/- 1.79 SD duration of pyrexia, bacteraemia: 3 (10%)** **Wound infection 1 (3.33%), pneumonia: 3 (10%), UTI: 2 (6.67%)** **Incidence of diarrhoea: 8 (26.7%), abdominal distention: 9 (30%)** **Control:** **4.80 days +/- 2.34 SD duration of pyrexia, bacteraemia: 9 (30%), wound infection 1 (3.33%), pneumonia: 5 (16.7%), UTI: 2 (6.67%), incidence of diarrhoea: 16 (53.3%), abdominal distention: 13 (43.3%)**
Zhang	**RCT**	**Total: 60** **Probiotic: 30** **Control: 30**	***B longum, L acidophilus* and *Enterococcus faecalis*.**	**Probiotic:** **Bacteraemia: 2 (6.7%), septicaemia: 1 (3.3%), pneumonia: 1 (3.3%), intra-abdominal abscess: 2 (6.7%), surgical site infection: 1 (3.3%)** **anastomotic leak: 0, intestinal obstruction: 3 (10%)** **Control:** **Bacteraemia: 9 (30%), septicaemia: 8 (26.7 %), pneumonia: 4 (13.3%), intra-abdominal abscess: 1 (3.3%), surgical site infection: 4 (13.3%), anastomotic leak: 2 (3.3%), intestinal obstruction 6 (20%)**

Abd El-Atti [30]	Case report	1	Multispecies	0 AE
Bellette [31]	Case report	1	Colotium (ADVITEC)—Culture showed growth of *Candida pelliculosa*, *Candida krusei, A. corymbiferaand Aspergillus flavus.*	Appendicitis and liver abscesses
Cesaro [32]	Case report	1	*Saccharomyces boulardii*	*Saccharomyces cerevisiae* fungaemia
Chitapanarux [22]	RCT	63 (placebo = 31; probiotics = 32)	*Lactobacillus acidophilus* and *Bifidobacterium bifidum (*Infloran®*)*	0 AE
Delia [23]	RCT	482 analysed (placebo = 239; probiotics = 243)	VSL#3 (multispecies)	0 AE
Giralt [12]	RCT	85 (placebo = 41; probiotics = 44)	*Lactobacillus casei DN-114* 001, *Streptococcus thermophiles* and *Lactobacillus delbrueckii subsp. bulgaricus*	0 AE
Henry [33]	Case report	1	Saccharomyces boulardii (Perenterol)	*Saccharomyces cerevisiae* found on blood cultures
LeDoux [34]	Case report	1	*Lactobacillus acidophilus* but not clear if additional organisms	Persistent *Lactobacillus acidophilus bacteraemia* on serial blood cultures for 3 days
Liu [25]	RCT	100 analysed (placebo = 50; probiotics = 50)	*Lactobacillus plantarum, Lactobacillus* and *Bifidobacterium longum*	0 AE
Malkov [35]	Case series	10	Bacillus oligonitrophilus KU-1	5 potential AE- Sicchasia (patient withdrew), blood pressure rise ×3 (patients' probiotics paused), ICP gain
Mehta [36]	Case report	1	Unclear but did	*Lactobacillus acidophilus* on blood cultures—though

			contain *Lactobacillus acidophilus*	not clear to tell if symptomatic
Naito [37]	RCT	202 analysed (group without probiotics = 102; group with probiotics = 100)	*Lactobacillus casei*	126 AE in group without probiotics; 80 AE in group with probiotics – unclear how many individuals these were distributed over. Wide range of gastrointestinal and urinary symptoms - unable to differentiate from malignancy (transitional cell carcinomas) or chemotherapy
Oggioni [38]	Case report	1	*Bacillus subtilis* spores *(Enterogermina)*	Blood cultures positive for *B. subtilis*
Osterlund [26]	RCT	148 (group without probiotics = 97, group with probiotics = 51)	*Lactobacillus rhamnosus GG*	No probiotic = 2 of 51; probiotic = 9 of 97 all cases of neutropenic infection (but no growth of Lactobacillus in blood cultures)
Sharma [27]	RCT	188 analysed (placebo = 95, probiotic = 93)	*Lactobacillus brevis CD2*	Placebo group = (7 × grade II dysphagia, 6 × grade II nausea and vomiting) + 1 died after developing grade IV neutropenia and sepsis; probiotic group = 1 × grade II dysphagia; 1 × developed acute myocardial infarction after 4 weeks of anticancer therapy - all attributed to chemotherapy by authors
Urbancsek [28]	RCT	205 (placebo = 103, probiotic = 102)	Antibiophilus sachets (containing *Lactobacillus rhamnosus*)	Placebo = 2 × GI problems (mild to moderate), 1 × labial oedema; probiotic = 3 × GI problems (mild to moderate)
Wada [29]	RCT	40 (placebo = 22; probiotic = 18)	*Bifidobacterium breve strain* Yakult (BBG-01)	0 AE

RCT: randomised-controlled trial

Table 10: Summary of Loke quality assessment of adverse events of studies identified during the update (85)

AE: Adverse event

Authors	Study type	Definitions of reported AE stated?	How were AE data collected	Any patients excluded from the adverse effects analysis?	Numerical data by intervention group	Categories of AE reported	Reporting of important or serious effects	Definition stated?	Methods for monitoring AE reported?
H Ekert, I H Jurk et al	RCT	Y	Spontaneous	Unclear	Y	New fever Positive culture	Unclear	Appearance of fever >38 persistence of symptoms of infection present at entry into study In all patients with temperature >38 on 2 consecutive 3 hour readings	No
K Kotzampassi, G Stavrou, G et al	RCT	Y	Prospective monitoring	Y	Y	Any major complication Any infectious complication Pneumonia Surgical site infections UTI Bacteraemia Severe sepsis Anastomosis leakage Need for mechanical ventilation	Y	Exact definitions not specified but outcomes to reported stated in outcomes	Yes

Liu ZH, Huang MJ et al	RCT	Y	Prospective	N	Y	Central line infection Pneumonia UTI Post-operative pyrexia Diarrhoea Incidence Positive bacterial cultures	Y	No clear definitions given (apart from bacterial cultures	Unclear
Liu Z, Li C et al	RCT	Y	Spontaneous	N	Y	Infection Septicaemia Diarrhoea Death Side effects of probiotics	y	Categories of adverse events not defined	y
M Mego, J Chovnec et al	RCT	Y	Prospective	Y	Y	Enterocolitis Bloating	Y	Some categories have not been defined e.g. pneumonia Definition of bacterial infection and post op fever given	N
M Mego, K Koncekova et al	Cohort	Y	Patient check list	n	Y	Infection Septicaemia Diarrhoea Death Side effects of probiotics	Y	According to Primary endpoint of this study was a prevention of grade 3/4 diarrhea according to CTCAE, Version 4.1. Secondary endpoints included the following: prevention of any grade of diarrhea,	N

								number of patients with any grade gastrointestinal symptoms and number of patients with any grade 3 or 4 toxicity or SAE related toxicity.	
Y Yang, Y Xia et al	RCT	Y	Routine	Unclear	Y	Infection Fever Neutropenia There were 14 microbiologically documented infections; bacteraemia caused by coagulase-negative staphylococci was the most frequent. Four patients experienced pneumonia, and two had perianal infection. Two patients experienced septic shock with a need of vasoactive support. There were no treatment-related deaths. Only two patients (14%) had mild diarrhoea (grade 1) during the treatment. Three patients (21%) experienced enterocolitis (two grade 2 and one	Y	Toxicity was graded according to NCI-CTC (version 2.0) criteria [21].	Y

						severe grade 3). "			
JW Zhang et al	RCT	Y	Prospective	Unclear	Y	Infectious complication and non-infectious complications	Y	Defined as section as observation of post-operative complication but not clear where definitions came fro	N

4 Mucositis and infection reduction with liquid probiotics in children with cancer: a randomised-controlled feasibility study (The MaCROS study)

4.1 Introduction

The systematic review reported in the last chapter demonstrated there were insufficient studies to assess the true effect of probiotics in people with cancer, particularly in children. Meta-analysis suggested that probiotics may be beneficial, but further studies were still required.

This chapter outlines and justifies the decision to undertake a randomised-controlled feasibility trial (the MaCROS study). The aim of this study was to explore the feasibility of conducting a randomised controlled trial (RCT) to investigate whether the use of probiotics could prevent or reduce mucositis and infection in children diagnosed with cancer. Participants in the feasibility study included children diagnosed with cancer receiving chemotherapy that may cause mucositis. The participants taking probiotics were compared to those taking a placebo supplement. Results are reported in following chapter (part 2) and is reported according to the CONSORT 2010 statement for feasibility and pilot studies (89).

4.2 Part 1: Setting up the MaCROS study

4.2.1 The rationale for undertaking a feasibility study

Randomised control trials (RCTs) are prospective studies that measure the effect of a treatment or intervention. RCTs are considered the gold standard for evaluating the effect of an intervention because they reduce selection bias through the random allocation of patient characteristics in a two-arm intervention (91). However, RCTs sometimes fail. Some reasons why an RCT may not work include:

- Researchers may be interested in a research question, but this may not be of interest to the patient with the relevant condition
- There could be difficulties implementing the protocol
- There could be a problem implementing allocation concealment, randomisation and blinding successfully

- It may not be possible to recruit enough participants to the study to achieve adequate statistical power
- Patients may struggle to adhere to the requirements of participation

Due to the costly and time-consuming nature of RCTs, it is crucial to identify any potential issues prior to conducting the study. These issues can be identified by undertaking a feasibility study. A clinical trial feasibility study is a research method for determining whether it is appropriate to undertake a larger study. Feasibility studies do not investigate primary outcomes but appraise important and essential parameters required to undertake a large adequately powered study such as an RCT.

4.2.2 Why is this feasibility study required?

The systematic review and meta-analysis presented in chapter 3 highlighted the need for further studies to investigate the use of probiotics in people with cancer, particularly in paediatric patients. Undertaking an RCT to explore this further would be very costly. Therefore, a feasibility study (the MaCROS study) was developed to investigate whether such an RCT could be undertaken successfully.

4.2.3 Approach to developing the MaCROS study

It was determined that a pragmatic clinical trial, rather than an explanatory trial would be more appropriate for the MaCROS study. Whilst an explanatory trial evaluates the efficacy of an intervention in an idealised setting, a pragmatic trial evaluates the intervention in everyday clinical setting for its applicability i.e. 'does this intervention work in real life?'(92).

The primary aim of this study was to explore the feasibility of undertaking a large and adequately powered RCT. For this reason, the secondary endpoints reported (for example the incidence of diarrhoea between the probiotic and placebo arm) were not adequately powered. These findings should, therefore, be interpreted with caution.

4.2.4 Developing the MaCROS study protocol

Once the primary aim of the MaCROS study was decided, the study protocol was designed to capture the necessary information whilst meeting the guidance of:

- the CONSORT 2010 statement for feasibility and pilot studies (89),
- ethical guidance issued by the Health Research Authority (HRA) (93),
- National Institute of Health Research (NIHR) (94)
- Leeds Teaching Hospital Trust research and innovation guidance (95).

The protocol was developed with expert input. This included health care professionals such as senior clinicians, dieticians and play therapists, senior academics and the University of Leeds ethics team and sponsors. The final version of the protocol can be viewed in appendix 2.1.

4.2.5 Deciding which probiotic to use in the MaCROS study

The systematic review undertaken prior to the development of this study identified significant heterogeneity between the type of probiotic strains used in the different studies, and that there was a particular lack of information about the strains used in paediatric studies. For the MaCROS study, a number of factors were considered prior to deciding which type of probiotic to use:

- Bacterial diversity: probiotics with more than one strain of bacteria were identified to reflect the bacterial diversity of the gastro-intestinal tract better.
- Mode of ingestion: children may struggle with swallowing tablets. A liquid option was sought, as this also gave the option of delivery via nasogastric tubing.
- The probiotic company's previous experience with research: as the MaCROS study was intended as a double-blind randomised controlled trial, it was preferred to have a company with previous experience of undertaking clinical trials and randomisation and supplying a placebo.

Following these criteria, the probiotic company Symprove was identified.

4.2.6 Symprove liquid probiotic

Symprove (Symprove Ltd, Farnham, Surrey, UK) is a liquid probiotic that contains four strains of bacteria with a total of 109 colony forming units:

- *Lactobacillus rhamnosus* NCIMB 30174,
- *Lactobacillus plantarum* NCIMB 30173,
- *Lactobacillus acidophilus* NCIMB 30175,
- *Enterococcus faecium* NCIMB 30176

In a water-based suspension of barley extract. Symprove is classified as a food supplement under EU law. It is hypothesised that suspension of the barley extract in water provides acid protection and a nutrient source for the bacteria when compared to freeze-dried probiotic formulations. Therefore a greater number of bacteria survives the transit through the human gastrointestinal system. This results in a higher number of probiotic bacteria to colonise in the colon (96). The

colonisation of bacteria from a probiotic supplement is believed to increase the secretions of anti-inflammatory cytokine interleukins (IL) such as IL-10 and IL-8, T-regulatory cells, and reduce interferons. These inhibit the development of oral and intestinal inflammation, and therefore mucositis (97).The use of Symprove in RCTs has been investigated in conditions affecting the gastrointestinal system, including inflammatory bowel disease, irritable bowel syndrome and diverticular disease.

4.2.6.1 Symprove in adults

Data suggests that Symprove reduces intestinal inflammation in patients with ulcerative colitis (96), and the frequency of diarrhoea and mucorrhoea in patients with diverticular disease (98). Another RCT demonstrated that the use of Symprove resulted in lower symptom severity in participants with irritable bowel syndrome (IBS) when compared to placebo (99).

4.2.6.2 Symprove in paediatrics

Symprove has been approved for use in children under EU law, and Symprove encourages the use of the liquid probiotics in paediatrics. However, dosing varies in younger children (20ml for those under the age of 4 and 0.5ml/kg for children aged between 4-8 years). There have been no paediatric clinical trials investigating the use of Symprove. Therefore the MaCROS study was the first trial exploring the use of Symprove in children with cancer. It was believed the liquid and flavoured formulations would improve compliance in children compared to tablets or freeze-dried formulations. As the use of Symprove has been investigated in other gastrointestinal disorders, it was an appropriate intervention to explore in children with cancer who are at risk of developing mucositis.

4.2.6.3 Safety of Symprove

Liquid probiotics have been reported as safe in previous studies (n=197 patients), and there have been no reports of unexpected serious adverse events attributed to Symprove (96, 99). However, consumption may be associated with nausea and reflux (99). As previously reported, in rare cases probiotics can be associated with infections in immunocompromised patients.

4.2.7 Patient information leaflets

The patient information leaflets designed were tailored to meet the requirements of children, teenagers and young people who were to participate in the study. Children and young people under the age of 16 may not have competence to understand a study, whilst young people above the age of 16 years are presumed to have

competence. Therefore, the leaflets were designed to reflect the different levels of understanding in children. Leaflets were created for the following ages:

- Children under the age of 10
- Children and teenagers aged 10-16 years
- Teenagers aged 16-18 years
- Parents/guardians of participants

Images used in the patient information leaflets were taken from Shutterstock (100).

4.2.7.1 Children under the age of 10

This patient information leaflet was designed with the intention of making the information engaging and relevant to young children. The Give a Duck charity donates a “Chemo Duck” to every child diagnosed with cancer in Leeds (101). Chemo Duck is a soft toy that represents experiences that a child diagnosed with cancer may go through. Chemo Ducks have central lines, a bandana for hair loss and hospital attire. This soft toy is given with the intention of alleviating the fears and anxiety children may have by introducing them to the concepts of cancer treatment (appendix 2.2) through play therapy.

4.2.7.2 Children and teenagers aged between 10 and 16

This leaflet was designed to deliver the relevant information in an engaging and relevant way for children and teenagers of this age range. Information was delivered using language that can be understood with engaging pictures taken from Shutterstock (appendix 2.3).

4.2.7.3 Teenagers and young adults aged between 16-18

As participants of this age range are expected to consent for themselves, the information leaflet was almost identical to the leaflets designed for parents and guardians. The only difference was language i.e. ‘you’ vs ‘your child’ (appendix 2.4).

4.2.7.4 Parents and guardians

This leaflet was designed to deliver the necessary information for parents and guardians to enable informed consent (appendix 2.5).

4.2.8 Consent of children and young people in clinical trials

There are a number of legal and ethical issues to consider when approaching children and young people to participate in research. The requirements for consent in these cases will depend on the type of study occurring and where in the UK it is taking place

(for example, Scotland or the rest of the UK). Clinical trials can be classified as clinical trials of an investigational medicinal product (CTIMP) or Non-CTIMP.

4.2.8.1 Clinical Trials of an Investigational Medicinal Product (CTIMP)

A CTIMP is a clinical trial that evaluates the safety or efficacy of a drug (known as an Investigational Medicinal Product or IMP) or obtains other relevant information such as how it is absorbed and metabolised.

Young people above the age of 16 are presumed to be capable of giving their own consent to participate in CTIMPs.

Children under the age of 16 are prohibited from giving consent to participate in a CTIMP and consent must be given on behalf of a child/ young person by:

- A parent or someone with parental responsibility
- A personal legal representative (only when someone with parental responsibility cannot be contacted prior to the proposed inclusion of a child/young person because of the urgent nature of treatment)
- A professional legal representative or a nominated person who is independent of the study

Children and young people should participate in decision making and be given information about the trials which is understandable to them.

4.2.8.2 Non-CTIMP trials

Non-CTIMP trials which do not involve an IMP as defined by the Medicines and Healthcare products Regulatory Agency (MHRA), do not fall within the requirements of the Medicines for Human Use (Clinical Trials) Regulations 2004. In England, Wales or Northern Ireland there is no statute which governs a child's right to consent in non-CTIMP trials and because of this both common law (derived from judicial decisions) and case law (collection of past legal decisions written by courts and tribunals) are considered and applied. Common law presumes that young people above the age of 16 are typically competent to give consent to treatment. Case law proposes that if a young person has sufficient understanding of the law to understand what is proposed and weigh up the information to reach a decision, he or she can give consent to treatment – for example, Gillick competence [ref]. In the absence of law related explicitly to non-CTIMP research, it is assumed that these principles also apply to consent for research in those under 16 years of age. Young people who are under the age of 16 who are competent to understand and weigh information to reach a decision do not need consent from a parent/responsible person.

It is good practice to involve families in the decision-making process whilst respecting the privacy of the young person. Therefore, parents of young people under the age of 16 were asked to give consent unless the young person explicitly stated they do not want this (appendix 2.6). All children and young people under the age of 16 were given the opportunity to assent (appendix 2.8). It is also good practice to supply information about the study (93). Competent young people above the age of 16 were required to give their own consent (appendix 2.7).

The MaCROS study was classified as a non-CTIMP clinical trial. To reflect the good practice that is recommended by the HRA, parental or guardian consent was required on behalf of all young people under the age of 16 unless there was an explicit request from the young person, and the young person was offered the opportunity to sign an assent form.

The consent and assent forms covered the necessary information for all aspects of the study. This included seeking permission to share information with the GP (appendix 2.9), storage of information and data after the study closed, dissemination of information and permission to be approached to participate in an interview (appendix 2.18).

4.2.9 Developing the patient diaries

The patient diaries were developed with the intention of capturing data to address the aims and objectives of the feasibility study. Where possible, validated questionnaires were identified and used to capture this information. For example, the Bristol Stool Chart (102) was used to describe stool consistency, and the Children's International Mucositis Evaluation Scale (ChIMES) (103) was used to describe oral mucositis. Currently, there is no validated tool to assess gastrointestinal mucositis in adults and children. Because of this, some questions were adapted from other validated tools such as ChIMES to answer questions about nausea and vomiting, and other features of gastrointestinal mucositis. Once the questions required to capture the necessary data were formulated, they were applied to a patient-friendly diary with the aim of making them easy to understand and quick to use.

Participants or their parents/guardians had the option of completing one of two diaries:

- A paper booklet
- An online diary that could be accessed using a web-app

Prior to the start of the MaCROS study, patients and parents within the department were asked to review the two types of diaries and provide feedback on what required

changing. Questions asked in the paper booklet and the online diary were identical and included questions to assess nausea, vomiting, diarrhoea, pain and oral mucositis.

4.2.9.1 Validated measurements tools used in the patient diary

The use of validated tools to capture information is vital for undertaking a randomised controlled trial. These tools may use subjective reporting (e.g. self-reporting) or proxy-subjective reporting by the parents of participants. Self-reporting has been increasingly recognised as the gold standard for collecting information on subjective information in paediatric haematology and oncology patients. This includes health-related quality of life and symptom burden questions. Reporting by a health care professional may not appropriately reflect symptoms that may be distressing to the patient. For example, whilst the visible appearance of an oral ulcer may be correctly reported by a health care professional, the same tool can under-report other unobservable symptoms, for example, nausea or pain. Previous studies have reported that clinicians may under-report the prevalence and severity of subjective symptoms when compared to patient self-reporting (104).

Subjective symptom reporting by parents on behalf of their children is known as ‘proxy reporting’. Previous studies have reported fair to moderate agreement between children and parent-proxy reporting. This suggests that parent-proxy reporting may not accurately reflect the child’s perspective (105).

A few subjective mucositis scales have been developed and validated for oral symptoms in adults. These include Patient-Reported Oral Mucositis Symptom scale (PROMS) (106) and the Oral Mucositis Weekly Questionnaire - Head and Neck cancers (OMWQ-HN) (107). In paediatrics, the Children’s International Mucositis Evaluation Scale (ChIMES) can be used by children aged 8 and above to assess symptoms of mucositis (103), with validated parent-proxy reporting for children under the age of 8. ChIMES focuses on the functional elements using simple questions and pictures of facial expressions that may reflect symptoms that the child experience.

However, there are no validated subjective reporting tools to assess gastrointestinal mucositis. Therefore, in the MaCROS study, tools that had been validated to assess oral mucositis were modified to enable assessment of gastrointestinal mucositis (108). A modified version of ChIMES was used to capture all necessary information, adding closed questions with multiple-choice options, and the Bristol stool chart to describe stool consistency using pictures.

4.2.9.2 Web-app

A web-app was developed for children, young adults and their parents/guardians to use, in order to increase the likelihood of compliance with reporting symptoms. The web-app is an electronic version of the paper diary. This is described in further detail in appendix 2.10.

Traditionally, the capture of self-reported information has been obtained from the use of paper patient diaries, questionnaires, or face to face encounters. However, paper diaries have the risk of participants completing data for multiple days at once, which can result in reporting and recall bias. They can also be misplaced, resulting in missing data. Electronic methods for self-reporting are increasingly used in research because some believe it can improve compliance, increase the amount of information which is provided by the patient, and has a higher acceptance rate by respondents (109). Whilst recall bias can occur with both paper and electronic diaries, the use of push notifications in electronic apps is thought to reduce this because they act as a reminder to the participants. As a result, the participants will be prompted to complete the relevant section when the information is required (110).

4.2.10 Data collection and analysis: protection and confidentiality

In the MaCROS study, data was collected using:

- Paper (e.g. clinical notes, consent forms, patient diaries)
- NHS electronic records
- Internet and software (web-app, encrypted audio recordings)

Some data was sensitive and had patient-identifiable and sensitive information. Because of this, methods to preserve and protect the anonymity of participants were implemented.

Data with identifiable patient information was accessed only on an NHS password-protected computer. Data transferred to the University of Leeds servers were anonymised, so that patient information was unidentifiable. Only the direct care team and researchers directly involved in the study had access to participants' personal data. Monitors and auditors from NHS R&D offices and regulatory inspectors may also require access to patients' clinical notes to verify or cross-check information. This information was provided in the information sheet to parents and participants aged 16 years and over, and only participants and legal guardians who signed the consent form were included in the study. Individuals who had access to participants' personal data were required to have an appropriate professional background and access to direct care.

4.2.10.1 Paper records

Consent forms/ participants diaries were stored in a locked filing cabinet in Martin Wing (offices for the paediatric haematology/oncology department) in LTHT. Only members of the research team had access to this filing cabinet. Written clinical records were accessed on the ward by a healthcare professional who was also a member of the research team (H.H). Data collected from written clinical records were transferred to an excel sheet that was accessed only on an NHS password-protected computer.

Participants who chose to decline to participate in the MaCROS study were invited to complete an anonymous questionnaire explaining why they chose not to take part in the study. Once transcribed, the anonymous questionnaires were destroyed. Participants were required to avoid mentioning any personally identifiable information.

4.2.10.2 Data collection and analysis: electronic records

Information was collected at Leeds Teaching Hospital trust on NHS password-protected computers.

An electronic database was created using a spreadsheet to store the patient's name, NHS number, unique randomisation number, age, sex, diagnosis/chemotherapy/course of treatment, sex and any relevant clinical information pertaining to hospital admissions (e.g. duration of stay, neutrophil count, blood culture results). Once this was completed, a duplicated spreadsheet was created, and the name, NHS number, and randomisation number were removed, leaving only the anonymised patient data.

Results (without any personally identifiable data) were emailed to the chief investigator (HH) using the encrypted and password protected NHS.net email account computer.

Information was then transferred to a database on the university M drive, a secure, password-protected, University of Leeds server. Data was analysed only on an NHS computer.

Strategies to ensure data protection of recorded interviews were also implemented. Only encrypted University of Leeds or NHS audio recorders were used, and it was required that participants do not refer to any patient identifiable data. The information was then transcribed onto a word document (identifiable only by the randomisation number). Once the transcribing was completed, the audio recording was deleted.

4.2.10.3 Web-app use: security and data protection

Data provided by participants in the web-app was identified only by their unique identification number, and there was no identifiable personal information. Information was downloaded to an excel spreadsheet and was accessed and analysed only on an

NHS password-protected computer. No identifiable data from the web-app was kept locally on laptops or computers.

The web-app was hosted by Amazon Web Services, one of the world's best providers with a high level of security.

4.2.10.4 Long-term data storage

As a PhD research project, raw datasets need to be held for a minimum period of 5 years after completion due to University regulations. The data, with consent forms, may also be needed for further follow-on studies evaluating longer-term outcomes. As such, the data will be stored for a total of 10 years. If appropriate, future studies would be submitted for their own ethical approval.

4.2.11 Patient and public involvement

Patient and Public Involvement (PPI) in research is described as research carried out with input from members of the public (in this case, patients). It is increasingly recognised that using PPI in the early stages of a study can optimise the impact and relevance of research (111). Feedback received from PPI early in the study design was used to identify and address issues that may impact the participant's experience, and consequently, their recruitment. Feedback from PPI can be used to revise aims, objectives, data collection and collection of outcome measures.

In the MaCROS study, PPI included children, teenagers and young people who are patients at the paediatric haematology and oncology department and/or their parents or guardians. Several children, young people, and their parents were approached to give feedback on the consent forms, PILs and patient diaries. This was to ensure they were easy to understand, and clearly explained participants' potential involvement in the study. Feedback involved:

- Parents giving feedback on the study aims and objectives
- Whether they would consider participating in a future study
- Voting for their preferred study short name acronym (MaCROS was the most popular)
- Giving feedback on the patient information leaflets and diaries that have been developed

We planned to use the feedback from patients and families to make changes which would make participation more engaging and user-friendly. However, the feedback received was positive, and no changes were needed.

4.2.12 Healthcare professional involvement

A play specialist (N.B) contributed to the revisions of the patient information leaflet for young children. Most suggestions focused on altering the wording and formatting to make the leaflet more engaging. All suggestions by N.B were included in the final version of the patient information leaflet. Furthermore, a dietician (E.W) with research experience within the topic of mucositis in oncology patients (112) highlighted the need to add a section about confidentiality in the patient information leaflets.

The study proposal was presented at the Experimental Cancer Medicine Centre meeting in January 2018, and the local Haematology and Oncology department at Leeds Teaching Hospital Trust. Queries and feedback were considered during revisions of the protocol prior to the submission of ethical review.

4.3 Part 2: Methodology

4.3.1 Introduction to the MaCROS study methodology

The previous sections discussed the justifications and the approach for developing the MaCROS study. The following section provides an overview of the study methods. Greater detail is provided in the MaCROS study protocol included in appendix 2.1.

4.3.2 Aim

This study aimed to evaluate the feasibility of an RCT investigating the efficacy of liquid probiotics in preventing or reducing mucositis and infection in paediatric oncology patients undergoing treatment regimes likely to cause mucositis.

4.3.3 Objectives

The primary objectives of this study were to determine:

1) Whether it is feasible to recruit children to a study when they are diagnosed with cancer and are at risk of developing mucositis
2) The completion rates of participants taking the liquid probiotic/placebo for 2 weeks
3) The completion rate of the symptom diary (paper/web-app) by participants or legal guardians recording the symptoms of nausea, vomiting, diarrhoea, oral mucositis and abdominal pain from the start of chemotherapy up until 21 days
4) Provide preliminary information on the health economics surrounding the costs and benefits of the intervention

Secondary objectives included:

1) Evaluation of the research protocol: by exploring the barriers and facilitators of the primary and secondary outcomes, and whether the protocol is pragmatic
2) Barriers to complying with the protocol
3) Evaluation of the outcomes intended to be assessed in an RCT. This could have included, but was not limited to, the incidence, severity and duration of diarrhoea and infection in both groups; the incidence of nausea, vomiting, oral mucositis; use of analgesia; and evaluation of hospital admissions.

4.3.4 Methods

The MaCROS study was registered prior to commencement (ClinicalTrials.gov Identifier: NCT03785938 (active), IRAS PROJECT ID: 246313, CPMS ID: 40800). The MaCROS study protocol, PILs and consent forms for the parents/responsible carers of

the children participating in the study and children aged 16-18 years old are enclosed in appendices 2.1-2.8.

4.3.4.1 Trial design

This was a single-centre double-blind randomised-controlled feasibility study.

4.3.4.2 Study setting

This study took place at Leeds Teaching Hospital Trust, (LTHT) Leeds UK between May and November 2019.

4.3.4.3 Participants

Participants were recruited from the Paediatric Haematology and Oncology Department (inpatient and outpatient settings).

4.3.4.4 Eligibility criteria for participants

Patients treated on paediatric cancer protocols, receiving chemotherapy, or on regimens that were likely to cause mucositis. Examples of protocols are included in appendix 2.1.

4.3.4.4.1 Exclusion criteria

- Patients who had already started the course of chemotherapy
- Patients receiving radiotherapy or surgery alone
- Patients who had taken probiotic supplements in the month prior to starting their next course of chemotherapy
- Patients with confirmed immunodeficiency

4.3.4.5 Target recruitment

The recruitment target was between 20 and 40 patients over a six-month period. As this was a feasibility study, a power calculation was not required.

4.3.4.6 Ethical review

This protocol was approved by the UK National Health Service (NHS) Ethics Committee process (REC ref: 19/YH/0005, appendix 2.11) and MHRA who confirmed that in this study, probiotics are classified as a supplement and that this would be classified as a Non-CTIMP study (appendix 2.12).

4.3.4.7 Consent

Consent on behalf of children under the age of 16 was taken from their parents/legal guardian. Children under the age of 16 were invited to complete an assent form

(appendix 2.8). Children aged 16-18 years old with capacity were required to supply their own consent.

4.3.4.8 Interventions

Participants were required to commence the blinded liquid probiotic or placebo enterally; either orally, via nasogastric tube, or gastrostomy; from the first day of their chemotherapy/pre-stem cell transplant chemotherapy conditioning. They were required to take this daily for 14 days. The dose prescribed varied according to age groups:

- Under the age of 4: 20 mL once a day
- 4-8 years of age: 0.5mls/kg once a day
- Above the age of 8: 1ml/kg once a day

4.3.4.9 Randomisation: type

Simple randomisation was used due to the small number of participants recruited.

4.3.4.10 Randomisation: implementation

This was undertaken by the trials pharmacist at LTHT.

4.3.4.11 Allocation concealment

Healthcare professionals (except the trials pharmacist) and participants were blinded to the randomisation allocation.

4.3.4.12 Blinding

Patients, healthcare professionals (except the pharmacy department) and the research team were blinded to the type of intervention delivered (intervention or placebo). Packaging for both groups was identical, and this was completed by the liquid probiotic company.

4.3.4.13 Data collection

4.3.4.13.1 Patient diary

The diary included questions to assess nausea, vomiting, diarrhoea, pain and oral mucositis (using a modified version of the ChIMES (103)). Either the participant or legal guardian was requested to fill in the diary, daily, for a minimum of 21 days. If possible, the same person was required to fill in the diary throughout the 21 days. They were given the option to complete a paper diary (appendix 2.16) or use a web-app which was secure and accessible only by the participant's randomisation number, which was issued by the trials pharmacist (www.macrosstudy.com).

4.3.4.13.2 Investigation of febrile episodes/infection

Clinical records, including electronic and written records, were reviewed to investigate for any instance of febrile episodes or , for the duration of any fever/infection, and for the duration of hospital stay until the patient was afebrile for 48 hours (table 16).

4.3.4.13.3 Other data

Other relevant information, including the type of nutritional support, analgesia and duration of hospital stay was taken from clinical records. Data collected were anonymised and stored on data collection forms.

4.3.4.14 Data analysis

4.3.4.14.1 Statistical analysis

Data were entered into a secure, local, anonymised database and analysed using descriptive statistics, Student's t-test, Mann–Whitney U-test, and $\chi 2$ tests, for comparative, normal, non-normal, and categorical data respectively. Where possible, appropriate subgroup analysis was to be undertaken.

4.3.4.15 Evaluation of the MaCROS study

The MaCROS study evaluated:

- The feasibility of undertaking a large, adequately powered RCT
- The safety of MaCROS (use of probiotics/placebo and reporting of unexpected serious adverse events)

This study was evaluated using both quantitative and qualitative methods. Data relating to the timing of the return of patient diaries, department referral rate, recruitment rate and numbers lost to follow-up was recorded. Acceptability and tolerability of the treatment intervention was assessed through the completion rate of the probiotic/placebo course, use of the patient diaries, and exploration of the patient’s/parent’s study participation via interview.

4.3.4.15.1 Indications to consider stopping the feasibility study

- The occurrence of an unexpected serious adverse reaction that is attributed to the probiotic or placebo

4.3.4.15.2 Indications to consider not progressing to a full RCT (RED)

- Inability to recruit 10 participants within 6 months of the study opening
- Poor compliance with recording and returning patient diaries (less than 50%)

- Serious concerns identified during the qualitative analysis of participant's/legal guardian's interview

4.3.4.15.3 Indications to consider modifying the study (AMBER)

- Poor recruitment: fewer than 20 participants recruited
- Poor identification of eligible patients
- Problems with delivery/compliance (50-80% of intervention or placebo delivered)
- Poor compliance with recording and returning patient diaries (less than 80%)

4.3.4.15.4 Indications to continue the study without modification (GREEN)

- No issues implementing the study protocol
- An adequate number of participants identified and recruited within the 6-month period
- 100% compliance with the delivery of the intervention/placebo
- Greater than 80% compliance of recording and returning of patient diaries

4.3.4.16 Evaluation of participant/parent experience

As part of the MaCROS study evaluation, a section was planned in which participants and/or parents were invited to discuss their experiences of participation in the trials. It was planned that participants who agreed to take part could be interviewed over the phone or in-person (depending on preference) and the interview audio could be recorded. The interview included questions regarding recruitment, the process of gaining consent and randomisation, and experiences using the probiotic/placebo and patient diary. The effect of these experiences on adhering to the study protocol was considered. Information was collected using a recording audio device, transcribed, and evaluated using the framework approach(113). The information was planned to be used in the development of any future RCT.

Unfortunately, despite the planning, it was not possible to carry out this section as it was not possible to successfully recruit enough participants for this part of the study.

Table 11: Summary of the 'traffic light' approach for evaluating the MaCROS study.

		
Identifying serious concerns during the qualitative analysis of the participant's or legal guardian's interview.	Poor identification of eligible patients.	No issues implementing the study protocol.
Inability to recruit 10 participants within 6 months of the study opening.	Poor recruitment- e.g. fewer than 20 participants recruited.	An adequate number of participants identified and recruited within the 6-month period.
	Problems with delivery/compliance (50-80% of intervention or placebo delivered).	100% compliance with the delivery of the intervention/placebo.
Poor compliance with recording and returning patient diaries (less than 50%).	Poor compliance with recording and returning patient diaries (less than 80%).	Greater than 80% compliance of recording and returning the patient diaries.

4.3.4.17 Safety reporting

The research team were responsible for identifying any adverse events. Serious Adverse Events (SAEs) were to be reported to REC using the safety reporting form recommended by the Health Research Authority (http://www.hra.nhs.uk/resources/during-and-after-your-study/progress-and-safety-reporting).

Because this feasibility study was classified as a non-CTIMP study, the only reports that were considered as SAEs were:

- Related to the study (i.e. as a result of administering the Symprove or placebo)
- Unexpected (i.e. not listed in the protocol as an expected occurrence)

Serious Adverse Reactions (SARs) are: An adverse event that is both serious and, in the opinion of the reporting investigator, reasonably believed to be caused by the trial treatments based on the information provided.

The strategies in place for safety reporting and a list of expected and unexpected SARs are summarised in the study protocol enclosed in appendix 2.1.

4.4 Part 3: Results

4.4.1 Introduction to the MaCROS study results

This section will include:

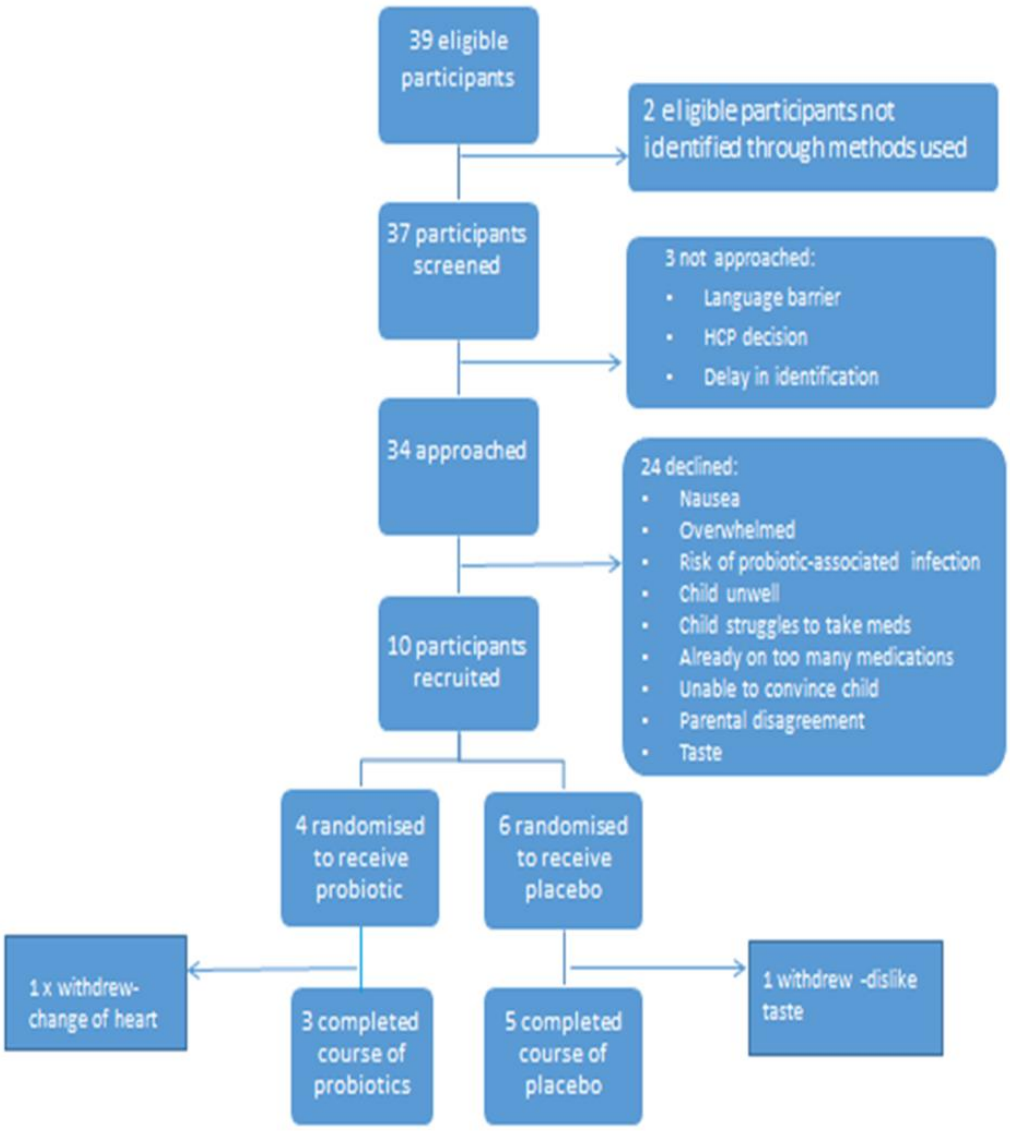
- The evaluation of the study
- Quantitative results
- Qualitative findings

Between May 2019 and November 2019, 39 children and young people were identified as eligible (diagnosed with cancer and at risk of developing mucositis).34 of these were approached to take part in the MaCROS study. 10 children (29.4% of those approached) were recruited. The ages of the participants recruited ranged from 1 year and 7 months to 15 years of age. The mean age of children recruited was 8 years and 7 months. Of these 10 participants, nine were diagnosed with solid tumours and one with acute myeloid leukaemia. Two of these nine participants received an autologous stem cell rescue for the delivery of high dose chemotherapy. Demographics are summarised in table 13.

Two participants (one placebo, one probiotic) chose not to continue participating because of the taste of the probiotic/placebo, and a change in desire to take the intervention/placebo. One of these participants submitted a partially completed diary and the other did not submit a diary upon completion.

Of these 10 participants, four were randomly allocated to the intervention group, and six were randomly allocated to the control group. Eight participants managed to complete greater than 80% of the total course of probiotic/placebo. The only reason documented that participants omitted a dose of probiotic/placebo whilst actively participating was being requested as ‘nil by mouth’ by clinicians. A consort diagram of this process is demonstrated in figure 8.

Figure 8: CONSORT diagram for the MaCROS study



4.4.2 MaCROS study evaluation

4.4.2.1 Setting up the study

Setting up the study was a multi-step process. This included:

- Developing the study protocol
- Identifying and communicating with the company Symprove who supplied the probiotic and placebo
- Liaising with the study sponsors and legal departments at the University of Leeds
- Liaising with the research pharmacy team to register the study, establish how blinding and how randomisation of the probiotic/placebo will take place
- Applying to the NHS REC, NIHR portfolio, HRA and LTHT R&D department
- Attending an NHS REC research ethics committee review
- Delivering a presentation regarding the MaCROS study to health care professionals within the local department involved in the study, alongside regional, national and international presentations

4.4.2.1.1 Facilitators to setting up the study

The HRA, Yorkshire REC and Portfolio application have been streamlined to ensure the duplication of an application does not occur. The submission of the MaCROS study was approved by REC and HRA following minor amendments (appendices 2.10 and 2.13). This demonstrates that this study is feasible to set up at both a local and national level.

4.4.2.1.2 Barriers to setting up the study

The application submitted to the Yorkshire REC and HRA for the MaCROS study took a significant amount of time to be reviewed and processed. Because LTHT R&D were unable to review the study until HRA had approved it, the pharmacy research team were unable to authorise the delivery of the probiotic/placebo by Symprove and thus undertake their required quality checks for dispensing. This resulted in the MaCROS study opening later than anticipated. Whilst the study was ultimately set up, the lengthy multi-step procedure was a barrier to setting up a future multi-centre study in a timely manner.

4.4.3 Primary outcomes

4.4.3.1 Identification

Between May and November 2019, there were 39 patients who met the eligibility criteria, of which 37 were screened, 34 participants were approached, and 10 were randomised.

Participants were identified in the following ways:

- New patients who were added to department's weekly MDT meetings
- Patient clinic lists
- Inpatients
- From recommendation by research nurses
- Word of mouth- e.g. consultant identifying a participant

Staff (doctors, nurses and allied healthcare professionals) within the department were informed of the MaCROS study through educational sessions delivered at departmental meetings, and one-on-one conversations. This was undertaken to increase awareness within the department, such that they could answer questions from eligible participants and families.

Five patients were eligible to participate, but were not approached for the following reasons:

- We were unable to communicate with the parents without an interpreter. Unfortunately, the parents were unable to read English PILs and consent forms.
- A child had relapsed, and the parents were highly distressed (an HCP decision made not to approach).
- Eligibility was discovered by an alternative route from that described above (and therefore did not meet the eligibility criteria because of the delay).

4.4.3.2 Declining participation

24 out of 34 eligible participants who were approached declined to participate. Of these, some were approached only once and others multiple times depending on their unique circumstances and their desires to have further time to consider joining. Families were approached more than once if they gave their consent to being re-approached. Reasons for deciding to decline are summarised in table 12.

Parents/carers who declined participation were given the opportunity to fill in an anonymous questionnaire (appendix 2.17) as described in the methodology section, in their own time. This questionnaire was developed and included in the MaCROS study to help the evaluation of a possible RCT. Struggling to recruit enough children and young people in paediatric cancer trials is recognised as a barrier for achieving an adequately powered RCT. It was intended that the anonymous questionnaire would help identify barriers that may have prevented recruitment in the MaCROS study, and may prevent participation in a future study.

No one completed the questionnaire. Reasons for this included:

- Being too busy
- Forgetting to complete the form
- Losing the form
- Changing their mind about completing it

Eligible participants appeared to prefer giving opportunistic verbal feedback. In future feasibility studies, this information could be more routinely captured by developing the ‘decline’ questionnaires, and having the researcher complete that after the discussion with parents/young people.

Table 12 Verbal reasons why participation in the study was declined.

Reasons for declining after being approached once	Reasons for declining after being approached two or more times
"Worried about experiencing nausea"	*Parents were unable to convince child to participate.*
"Too much to deal with right now"	*Parent felt that their child already had too many medications.*
Worried about risk of probiotic-associated infection.	*Initially overwhelmed with the new diagnosis. Asked researcher to return later, and then declined.*
Struggling to persuade the child to take any oral medicines.	*Child too unwell.*
Child dislikes the taste of milk/yoghurts.	*Mother wanting to participate, father not happy to.*
Doesn't want to take 'gamble' of potentially receiving the placebo.	*Heard from another parent that the probiotic/placebo doesn't taste nice.*

4.4.4 Included participants

Ten eligible patients agreed to participate in the MaCROS study. Of these, 9 were diagnosed with solid tumours, and 1 was diagnosed with a malignant haematological condition. Two patients diagnosed with neuroblastoma were undergoing autologous stem cell rescue for high dose chemotherapy. Details of the anonymised demographic information is summarised in table 13.

4.4.5 Patients who withdrew participation

Two participants aged 12 (placebo) and 15 years (probiotic) respectively withdrew participation after one day. Both reported a dislike for the taste of probiotic/placebo and stated they were unable to continue with the full 14-day course. One participant returned the diary after completing the first page, and the other participant did not wish to complete any of the diary.

Table 13: Summary of demographics of patients included in the MaCROS study

Patient number	Age	Sex (Male = M / Female = F)	Diagnosis	Cycle/day	Consent	Date commenced	Paper/diary	Stopped further participation in MaCROS study
1	12 years	F	Ewing's Sarcoma	Last cycle	10/06/2019	10/06/2019	Paper	13/06/2019
2	13 years	F	Osteosarcoma	Cycle 6	02-Jul	04-Jul	Web-app	
3	7 years	F	Undifferentiated sarcoma	Cycle 2	02/07/2019	02/07/2019	Not completed	
4	12 years	F	HR NBL- HD chemotherapy and stem cell rescue	Day 0	04/07/2019	04/07/2019	Not completed	
5	1 year, 7 months	M	HR NBL	Day 20	04/07/2019	04/07/2019	Paper	
6	1 year, 7 months	F	HR NBL- HD chemotherapy and stem cell rescue	Day 0	04/07/2019	11/07/2019	Web-app	
7	14 years	M	AML	Cycle 1	04/09/2019	05/09/2019	Web-app	
8	3 years	M	NHL	Cycle 2	05/09/2019	09/09/2019	Paper	
9	15 years	M	Metastatic relapsed osteosarcoma	Cycle 1	09/07/2019	10/09/2019	Paper	11/9/19
10	8 years	M	Osteosarcoma	Cycle 3	19/11/2019	19/11/2019	Paper	

4.4.5.1 Facilitators to recruitment

87% of eligible participants were correctly identified in a timely manner using the methods described previously. 94% of those identified were happy to be approached for a brief introduction and discussion regarding participation in the MaCROS study. Most families showed initial interest in participating in the study.

4.4.5.2 Barriers to recruitment

4.4.5.2.1 Identification

The previous methods described did not identify 100% of eligible participants. Two participants were not appropriately identified in a timely manner, for the following reasons:

- Participants were not identified via review of MDTs and clinic patient lists
- Participants were not identified and conveyed to the research team
- Participants were not identified at the right time in their program of treatment, i.e. prior to the start of their next or final course of chemotherapy.

Specific reasons for not being identified at the right time include changes/delays to their start of treatment. For example, a number of those who were not appropriately identified were those undergoing allogeneic HSCT. This was because the HSCT may have been delayed (due to the child being too unwell) and the research team were not aware of the new date.

The responsibility of recruitment, and subsequently consent, was mostly undertaken by one member of the research team (but by two persons in total). This could have limited the number of eligible patients being identified in a timely manner. Delivering training to the broader medical team on the topics of recruitment, consent and prescribing of the probiotic/placebo could ensure a higher proportion of eligible participants are recruited in future studies.

4.4.5.2.2 Selection bias

Several eligible patients who were identified in a timely manner were not approached or re-approached due to explicit decisions made by the clinicians involved in their care. Whilst the reasons to not approach (or re-approach) patients may arguably be justified, it may have altered the sample of participants who were successfully recruited. Examples include:

- Not approaching patients because they have relapsed with incurable disease (although it is worth noting that a patient with a relapsed incurable disease was successfully recruited)
- Not approaching patients because their families are visibly upset (due to relapse/disease-progression/ severity of treatment-related morbidity)
- Not approaching patients who were newly diagnosed because clinicians felt patients and their family required space to process information

It was noted early in the study that patients who were eligible shortly after diagnosis (e.g. a patient diagnosed with B-ALL who is due to start induction) tended to decline participation because they felt distressed, overwhelmed, wanted to prioritise essential interventions for their child, or had already been approached to participate in a number of research studies and felt they had been 'overloaded' with requests. Therefore, a decision was made one month into the MaCROS study to not approach patients who were recently diagnosed with cancer.

These factors may have contributed to the recruitment of a greater number of patients experiencing fewer treatment-related morbidity side effects (i.e. those diagnosed with solid tumours vs those diagnosed with malignant haematological conditions).

4.4.5.3 Consent

Once patients and their families verbally consented to take part in the study, they then signed a consent form. Some families consented to participate in the MaCROS study but declined consent to participate in an interview to discuss their experience of participating in the study. Several parents mentioned they were only interested in taking the probiotic/placebo and did not have the time to take part in an interview. Five participants agreed to participate in an interview at the time of consent, but none were undertaken. Four participants did not reply to telephone enquiries to set a date. Date and time were agreed for one participant; however, they were not able to commit closer to the time. No teenage participant expressed a desire to sign the assent form. Formal consenting by parents was straight forward following a verbal agreement to participate.

4.4.5.3.1 Facilitators to the consent process

Patient information leaflets (PILs) were positively received. Most patients/guardians did not have any questions prior to consent because they felt the PILs clearly explained the risks, benefits and processes of the MaCROS study.

4.4.5.3.2 Barriers to consent

There were no barriers to the consenting process. In some instances, parents consented and expressed a desire to participate in the MaCROS study, but their teenage child did not. In these situations, the patient was not recruited. One particular case involved non-married cohabiting parents, where the father was not on the birth certificate. Whilst the mother was keen for her child to take part in the MaCROS studies the father was not. Following a discussion with the child's mother, a decision was made to respect the father's wishes (even though it was not required legally).

4.4.5.4 Prescribing, randomisation, allocation, concealment and dispensing

Prescribing of the probiotic/placebo occurred once written consent was obtained by a prescriber. This was a two-step process, including:

- Completing and submitting a form issued by the pharmacy team containing patient demographics, identification numbers, weight and allergies. Doses were calculated according to that specified in the protocol and the number of bottles that would be required. Only those with signatories on the MaCROS study delegation log were able to complete this form.
- Prescribing an anonymised clinical trials medicine on *Emeds,* the electronic prescribing system used at LTHT. This could be completed by any prescriber.

Simple randomisation was undertaken by the research pharmacy team at Leeds. The LTHT lead trials pharmacist (P.S.) was responsible and undertook randomisation. None of the research or health care team had access to the randomisation code used.

Symprove delivered the probiotics and placebos to the pharmacy department in boxes of unmarked 4x 500 mL bottles. Symprove and the pharmacy team liaised directly without input from the research teams to confirm which of the boxes contained the probiotic or placebo. Only the research pharmacy team knew allocation for each participant and ensured blinding took place. The probiotic/placebo was labelled as 'clinical trial medicine'. Those who dispensed the probiotic/placebo were not aware of which item the product contained. No member of the research team, healthcare professionals or participants were made aware of the allocation. The allocation was only revealed to the research team once the study had closed and the final patient had completed participating in the study.

4.4.5.4.1 Facilitators to prescribing, randomisation, allocation, concealment and dispensing

The prescribing, randomisation, allocation, concealment and dispensing were quick and easy to undertake. A prescription form was given to the local pharmacist who delivered the form to the pharmacy research team.

4.4.5.4.2 Barriers to prescribing, randomisation, allocation, concealment and dispensing

Completion of the form could only be undertaken by those whose signatures were on the delegation log (H.H. and B.P.). This limited when prescribing was able to occur, as it could only be undertaken if either member was present on hospital premises. This issue could be avoided in future studies by allowing all GCP trained prescribers within the clinical team to place their signature in the delegation log.

Submitting the prescription form, undertaking randomisation, and blinding allocation concealment could only occur during standard working hours by the research pharmacy team. If a patient was consented out of hours (evenings, weekends or bank holidays), delivery of the form could not take place until the next working day. Even if prescribing occurred in a timely manner, a delay in delivery of the prescription form could nullify the eligibility of the participant identified. However, this did not occur with the 10 participants who were recruited.

For the feasibility study, it was possible to undertake simple randomisation due to the small number of participants recruited. However, in a future multi-centre randomised controlled trial, stratified randomisation undertaken by a statistician within a Clinical Trials Unit is likely to be required.

4.4.5.5 Adherence to the probiotic/placebo

Of the ten participants who were recruited to the MaCROS study, four participants were randomly allocated to the intervention group, and six participants were randomly allocated to the control group. In total, two participants withdrew from the study (one probiotic and one placebo) within 24 hours of participating in the study.

4.4.5.5.1 Facilitators to adherence to the probiotic/placebo

80% of participants adhered to the full course of probiotic/placebo. It was successfully administered orally and via nasogastric tubing consistently for the 14-day course.

4.4.5.5.2 Barriers to adherence to the probiotic/placebo

Two participants (one probiotic, one placebo) withdrew from the MaCROS study. Both participants chose to withdraw as they were not able to tolerate the taste. A further

teenage participant disliked the taste and stated it made them feel nauseous, but that they chose to complete the course, because they understood the importance of participating in research trials to help future children diagnosed with cancer.

The volume of probiotic was mentioned by several parents who completed participation in the feedback given in the diaries. Some parents stated they struggled to deliver the volume required and that they felt using smaller volume and higher concentration would be easier to manage. One parent felt that distributing the volume throughout the day (e.g. twice a day instead of once a day) would be easier.

4.4.5.6 Data capture

Data was captured using paper and web-app diaries which were filled in by participants or their parents/carers. Seven participants opted to use the paper diary, and three opted to use the web-app (table 13). Only one participant chose to fill in the diary (web-app) themselves, and the other nine were completed by their parents/carers.

Three parents who chose to use the paper diary stated they would prefer to use the web-app but found the WiFi connection in the inpatient area unreliable. The other parents who chose to use the paper diary stated that the paper diary would be easier to use as they were not comfortable with technology.

The clinical notes and electronic records were recorded during inpatient stay for all 10 participants. Records were reviewed to identify and capture (i) information of febrile episodes, and (ii) infections, for (a) incidence and duration of fever/infection, and (b) duration of hospital stay.

50% of data collected from the diaries and electronic records were reviewed and confirmed by a second reviewer (L.S). Out of the 10 participants, seven partially completed the diaries. Four out of seven (57%) submitted paper diaries with partially completed data, and three out of three (100%) submitted partially completed data on the web-app. Three out of the seven participants who submitted data completed at least 80% of data for 14 days (duration of the course of probiotic/placebo). Only two participants completed 80% of the data required for the 21 days. No participant completed 100% of the information requested.

4.4.5.6.1 Paper diary

Four out of seven participants who chose to use the paper diary partially completed the questions and returned the diary. Two participants completed 80% or more data for 14 days, and the other two partially completed the data.

Three participants did not return a paper diary. Of these, two stated they had misplaced the paper diary. The third person stated they would submit it on their next clinic appointment but forgot to do so.

4.4.5.6.2 Web-app diary

All participants who chose to use the web-app partially completed data. One participant completed 80% of the information for the 14-day course of the probiotic/placebo and the other two participants submitted a partially completed diary via the web-app.

4.4.5.6.2.1 Facilitators for the capture of data (diaries)

The paper diaries were easy to use and understand. Parents who gave verbal feedback all stated the questions were easy to understand. All participants who used the web-app stated it was easy to navigate.

4.4.5.6.2.2 Barriers for the capture of data (diaries)

Participants stated they forgot to complete the diary on a daily basis. Parents stated this typically occurred when they were busy, and therefore at times they would complete data for several days in one go.

One parent gave feedback in their paper diary, stating they found it challenging to assess nausea and pain because their child was too young to communicate. Another parent stated they would have benefited from daily push notifications. However, participants/parents would have had to separately consent to be receiving reminders, and none of the three participants gave permission to do so. 30% of participants did not return their paper diaries. This is because they had either misplaced or forgot to return their diary.

4.4.5.7 Clinical data

100% of data was captured involving the inpatient stay using clinical information taken from written and electronic records into a pre-developed proforma, using a Microsoft excel spreadsheet. 50% of the data collected was verified by a second reviewer for accuracy.

4.4.5.7.1 Facilitators to data collection

Collecting data from written and electronic records and using the pre-developed proforma was undertaken with ease.

4.4.5.7.2 Barriers to data collection

Lack of documentation in clinical records was the main barrier involving data collection. For example, a repeat blood culture may have been undertaken, but the indication was

not specified. Therefore, whilst the proforma could capture the information as documented, it may not capture the full clinical picture.

4.4.6 Summary of primary outcomes

The MaCROS study protocol received ethical approval and successfully opened in May 2019. Between May and November 2019, 10 participants were recruited out of 39 eligible participants, of which 34 were approached. Simple randomisation, allocation concealment and double blinding successfully took place. Four participants were randomised to receive the probiotic and six participants received the placebo. Of these, eight participants (three probiotics, five placebo) completed the full course. Seven participants returned partially completed diaries (four paper, three web-app). No participant completed data for the full 21 days. Three out of seven participants (42.9%) completed 80% of the data for the duration of the 14-day course of probiotic. No patient who declined consent opted to fill the anonymous questionnaire, although many participants consented to give verbal feedback and discussion of their reasons. Five participants agreed to participate in an interview at the time of consent. However, four of these participants did not reply to enquiries. One participant agreed to a time and date but could not commit at the allocated time.

No unexpected serious adverse events were reported. A summary of the evaluation of the MaCROS study, using the traffic light system demonstrated in table 11, is summarised in table 14.

Table 14: Findings from evaluating the MaCROS study using the traffic light system

Aim	Result	Outcome
Implementing protocol	No issues	Proceed
Recruitment	10 participants recruited	Modification required.
Identification of eligible participants	Adequate identification of eligible participants	Proceed
Problem with delivery of intervention/placebo	No problems	Proceed
Adherence-probiotic/placebo	80% adherence with completing course	Proceed
Adherence-Completion/adherence of diary	Poor compliance with completion of diary/	Modification required

4.4.7 MaCROS study evaluation: intended outcomes to be assessed for a future RCT

4.4.7.1 Statistical findings

Statistical analysis could have included, but was not limited to; the incidence, severity and duration of diarrhoea and infection, the incidence of nausea, vomiting and oral mucositis, use of analgesia, total parental nutrition (TPN) and evaluation of hospital stay.

Incomplete data was a significant issue. Specific questions were left blank by patients even when responses were given for that day. Because of this, a descriptive analysis was undertaken with no attempts to impute missing data, and results should be interpreted cautiously.

It was anticipated that the MaCROS study could be used to calculate the sample size required for a future clinical trial at an appropriate power. Statistical power is defined as the probability that an outcome is not attributed to chance. To calculate the power of a study, three variables are required:

- The acceptable possibility of a false positive (type 1 error) occurring (which is usually set at 0.05)
- The magnitude and variability of effect in the population
- The size of the sample

Because the study had fewer than anticipated participants, and incomplete diary entries, it was not possible to reasonably calculate the standard deviation of symptom outcomes and estimate the possible magnitude and variability of effect. While historical data studies can be used to determine the treatment effect size, the systematic review and meta-analysis undertaken for this thesis identified significant heterogeneity between studies, meaning it is not possible to reasonably use these data Examples of heterogeneity identified included the type of probiotic used, outcomes investigated, and a lack of paediatric studies.

4.4.7.2 Diary results

Table 15 summarises the findings from the patient diaries. Seven out of 10 patients submitted partially completed diaries. The percentage of days filled (excluding those who did not return diaries) for the duration of the 21 days ranged from 4.8%- 90.5%. The median percentage of total diary completed was 46.9% (approximately 10 days of data). The participants were more likely to fill in the diary when taking the probiotic/placebo; the mean percentage of diary completed for the first 14 days was

64.2% (range 7.14-100%), and 5/7 (71%) participants who returned diaries completed more than 75% of data during the 14-day course.

Patient number	Probiotic/ placebo	Percentage of diary completed (21 days in total)	Percentage of diary completed 14 days	Median loose stool (range) per day	Median stool type per day (range)	Median score for nausea per day (range)	Median frequency of vomiting per day (range)	Median pain per day (range)	Median difficulty drinking (range)	Median difficulty swallowing (range)

Table 15: Findings from patient diary

1 *	Probiotic	4.8%	7.14%	0	1	3	0	3	1	1
2	Placebo	52.3%	78.5%	1 (0-5)	3 (1-5)	3 (1-5)	0 (0-0)	4 (0-5)	2 (1-4)	2 (1-4)
3	Probiotic	0	0	NA	NA	NA	NA	NA	NA	NA
4	Placebo	0	0	NA	NA	NA	NA	NA	NA	NA
5	Probiotic	61.9%	92.9%	1 (0-3)	6 (4-7)	Not documented	1 (0-2)	1 (1-2)	1 (0-3)	1 (0-3)
6	Probiotic	90.5%	100%	3 (0-10)	5 (4-7)	2 (1-3)	1 (0-3)	1 (0-3)	0 (0-2)	0 (0-2)
7	Placebo	4.8%	7.14%	7	4	1	Not documented	1	1	1
8	Placebo	61.9%	92.9%	3 (0-7)	6 (6-7)	2 (1-3)	1 (0-3)	1 (1-3)	2 (1-4)	3 (0-4)
9*	Placebo	0	0	NA	NA	NA	NA	NA	NA	NA
10	Placebo	52.3%	78.5%	1 (0-3)	4 (3-7)	2 (2-4)	1 (0-3)	1 (1-3)	NA	2 (1-4)

**patient stopped participation*

4.4.7.3 Inpatient admissions

Nine out of 10 participants had an in-patient stay. All data captured involving the inpatient stay was from clinical information, taken from written and electronic records, as previously described.

4.4.7.4 Pyrexia and infection

Table 17 presents a summary of the participants who were febrile (non-neutropenic), febrile (neutropenic) or had grown an organism from a blood culture without fever. Two of the three participants who were febrile but not neutropenic did not receive antibiotics. The participant who did was undergoing a high-risk procedure (autologous transplant). All three participants who developed febrile neutropenia received antibiotics. One participant who was not febrile received antibiotics following the recommendation from the microbiology team.

4.4.7.5 Other supportive care interventions

Table 18 summarises supportive care interventions delivered to the participants recruited to the MaCROS study. No participants were admitted to intensive care. Expected serious adverse events which occurred included a participant developing neutropenic enterocolitis, vaso-occlusive disease (VOD), post-transplant ileus and a *C.difficile* infection. 10 participants (100%) required antiemetics for nausea and seven participants (70%) required analgesia. Of these, two participants (two probiotics) required a Patient-controlled analgesia (PCAS). Four participants (40%, two probiotics, two placebos) required nasogastric tubes, and one participant in the placebo group required Total parental nutrition (TPN).

Table 16: Data taken from clinical and electronic records of those recruited to the MaCROS study

Patient no	Probiotic/ placebo	Age	Diagnosis	Inpatient (routine chemotherapy)	Hosp Admission/	Febrile	Febrile Neutropenic?	Positive blood culture?	Organism
1 *	Probiotic	12 years	Ewing's Sarcoma	Yes	No	No	No	N/A	
2	Placebo	13 years	Osteosarcoma	Yes	Yes	Yes	No	No	
3	Probiotic	7 years	Undifferentiated sarcoma	Yes	No	No	No	NA	
4	Placebo	12 years	HR NBL-HD chemo and stem cell rescue	Yes	NA**	Yes	Yes	No	
5	Probiotic	1 year, 7 months	HR NBL	Yes	No	No	No	Yes	*Streptococcus mitis/oralis. Streptococcus vestibularis* Streptococcus parasangui
6	Probiotic	1 year, 7 months	HR NBL- HD chemo and stem cell rescue	Yes	NA**	Yes	No	Yes	Gram negative bacilli
7	Placebo	14 years	AML	Yes	NA**	Yes	Yes	No	
8	Placebo	3 years	NHL	Yes	Yes	Yes	Yes	No	
9*	Placebo	15 years	Metastatic relapsed	No	No	No	No	NA	

Patient no	Probiotic/ placebo	Age	Diagnosis	Inpatient (routine chemotherapy)	Hosp Admission/	Febrile	Febrile Neutropenic?	Positive blood culture?	Organism
			osteosarcoma						
10	Placebo	8 years	Osteosarcoma	Yes	Yes	Yes	No	No	

*Withdrawn ** Inpatient until count recovered

Table 17: Results of participants who were febrile during hospital admission

Patient no	Probiotic/ placebo	Age	Diagnosis	Febrile Neutropenic?	Blood test results	Positive blood culture?	Organism	Additional information
2	Placebo	13 years.	Osteosarcoma	No	Neut 10.5x10^9/L WCC 11.5 x10^9/L CRP 41 mg/L	No		? Temperature related to mifamurtide Not commenced on antibiotics
4	Placebo	12 years.	HR NBL	Yes	Neut 0.02 x10^9/L WCC 0.04 x10^9/L CRP 244 mg/L	No		Treated for thrush (mouth swab *Candida albicans*) and post-transplant ileus (conservative management) Received Tazocin
5	Probiotic	1 year 7 months.	HR NBL	No	Neut 0.63 x10^9/L WCC 2.26 x10^9/L CRP not checked	Yes	Streptococcus mitis/oralis Streptococcus vestibularis Streptococcus parasangui	CRP not checked until noted to have positive culture result Not febrile or neutropenic. Cultures taken after patient vomited on himself and requiring a change of central line smart sites and routine cultures being taken Treated with vancomycin

Patient no	Probiotic/ placebo	Age	Diagnosis	Febrile Neutropenic?	Blood test results	Positive blood culture?	Organism	Additional information
6	Probiotic	1 year, 7 months	HR NBL	No	Neut 0.68 x10^9/L WCC 0.71 x10^9/L CRP not checked	Yes	Gram negative bacilli	Treated for neutropenic sepsis, neutropenic enterocolitis and VOD Unable to fully identify organism 97% similarity to proposed genus *Anaeromassillibacillus* Treated with tazocin and tobramycin then switched to Meropenem
7	Placebo	14 years.	AML	Yes	Neut 0.03 x10^9/L WCC 0.71 x10^9/L CRP 52 mg/L	No		No possible focus documented. Treated with Tazocin
8	Placebo	3 years.	NHL	Yes	Neut 0.09 x10^9/L WCC 0.14 x10^9/L CRP 21 mg/L	No	*C. diff* toxin positive stool	Loose stools- moderate severity CDT score. Commenced metronidazole for CDI and meropenem (penicillin allergic) for FN
10	Placebo	8 years.	Osteosarcoma	No	Neut 5.38 x10^9/L WCC 7.3 x10^9/L CRP 20 mg/L	No		Coryzal, NPA negative not started on antibiotics

Neut: Neutrophils WCC: White cell count CRP: C-reactive protein

Table 18: Additional symptoms and supportive care interventions

Patient no	Probiotic/ placebo	Age	Diagnosis	Nasogastric feeds	Required TPN	IV fluids*	Critical care admission	Antiemetics	Analgesia	Location of pain
1 *	Probiotic	12 years.	Ewing's Sarcoma	No		Yes	No	Yes	PCAS	Mouth Stomach
2	Placebo	13 years.	Osteosarcoma	No		No	No	Yes	No	NA
3	Probiotic	7 years.	Undifferentiated sarcoma	Yes		No	No	Yes	Dihydrocodeine	Not known
4	Placebo	12 years.	HR NBL	Yes	Yes	Yes	No	Yes	PCAS	Mouth, stomach
5	Probiotic	1 year 7 months.	HR NBL	Yes		No	No	Yes	Dihydrocodeine	Not known
6	Probiotic	1 year 7 months.	HR NBL	No		Yes	No	Yes	Oramorph	Not known
7	Placebo	14 years.	AML	No		No	No	Yes	Dihydrocodeine	Not known
8	Placebo	3 years.	NHL	Yes		Yes	No	Yes	Oramorph	Mouth, Throat

Patient no	**Probiotic/ placebo**	**Age**	**Diagnosis**	**Nasogastric feeds**	**Required TPN**	**IV fluids***	**Critical care admission**	**Antiemetics**	**Analgesia**	**Location of pain**
										and Tummy
9*	Placebo	15 years.	Metastatic Osteosarcoma	No		No	No	Yes	No	NA
10	Placebo	8 years.	Osteosarcoma	No		No	No	Yes	No	NA

4.4.7.6 Qualitative evaluation- feedback from the diary

Four participants recorded feedback in the paper diaries. Some feedback related to adherence with the probiotic/placebo and use of the patient diary. Table 19 summarises this feedback, which has been anonymised and paraphrased for confidentiality.

Table 19: Feedback delivered in diaries (both paper and web-app)

Probiotic/placebo	**Feedback**
Probiotic	*"X found it difficult to drink the sample as the smell is quite off-putting, we did get it down but I'm sure if it had a better smell the task would be easier"* *"From an adults' point of view, all the chemo and extra meds the kids have to then take another product that has such a bad smell and taste is hard for them"*
Probiotic	"*Y stopped eating-not sure if its due to feeling sick or not eating because of mucositis*" *"I don't think she had the trial medicine today as she had to stop any oral intake (bowels slowing down)"* *"Trial med not given as no oral/NG tube allowed"*
Placebo	*"Dose taken an hour later as Z had yoghurt with food at the time dose was due"* *"Being a baby, it is difficult to say how sickly Z feels so it's all a best guess"* *"Would be helpful to have reminders"*
Placebo	*"20 mL is too much to put down an NG tube in a small child"* *"Not eaten for a while"* *"Again 20 mL is too much volume, it makes him retch as you*

	put it down”

4.4.7.7 Economic evaluation

4.4.7.7.1 Study set up

No research costs other than funding for the author of this thesis was required to set up the MaCROS study. This study received NIHR portfolio funding, and this enabled direct access and support, from research nurses who work within the department, at no extra cost. A research nurse directly communicated with some participants, approached eligible participants to participate in the study, supplied PILS and collected diaries from participants upon completion.

The company Symprove supplied the probiotic and placebo to the MaCROS study free of charge and donated costs to the development of the web-app.

4.4.7.7.2 Inpatient stay

Due to the limited number of participants in the study, it was not possible to undertake formal economic evaluation (cost/benefit analysis). However, there were no unexpected serious adverse events and no prolonged hospital admissions due to the probiotic.

4.5 Summary

The MaCROS study protocol received NIHR portfolio status (appendix 2.15), ethical approval from the HRA (appendix 2.13), Yorkshire REC (appendix 2.11) and LTHT R&D (appendix 2.14) and opened to recruitment 23rd May 2019. Between May and November 2019, 39 eligible participants were found to be eligible, of which 34 were approached and 10 were recruited to the study. Simple randomisation, allocation concealment and double blinding were successfully implemented. Four participants were randomised to receive the probiotic and six participants received the placebo. Of these, eight participants (three probiotics, five placebo) completed the full course. Seven participants returned partially completed diaries (four paper diaries, three web-app). Of these, no participants completed data for the full 21 days. Three out of the seven participants (42.9%) completed 80% of the data for the duration of the 14-day course of probiotics. No patient who declined consent opted to fill the anonymous questionnaire, although verbal feedback was given at the time. Five participants agreed to participate in an interview at the time of consent, but no interviews occurred. Four participants gave feedback on the study in the supplied diaries.

Missing data was a significant issue. Select questions were left blank even when responses were given for that day.

Data was captured from clinical and electronic records for all participants recruited to the MaCROS study. Seven of these participants either developed pyrexia or were noted to have a positive blood culture result. Six participants (one probiotic group, five placebos) developed pyrexia. Three of these participants also had febrile neutropenia (three placebos) who were also classified as high-risk febrile neutropenia. Two participants (two probiotics) had positive culture results. One of these participants was noted to have a positive culture from a routine check despite not being febrile or febrile neutropenic. Organisms reported include an unidentified organism, *Streptococcus mitis/oralis*, *Streptococcus vestibularis* and *Streptococcus parasangui*. A participant in the placebo group with febrile neutropenia was also treated for oral thrush. A further participant in the placebo group developed a *C. difficile* infection (positive toxin) which required treatment with oral metronidazole.

There were no deaths, and no participants were admitted to intensive care. Expected serious adverse events which occurred included a participant developing neutropenic enterocolitis, vaso-occlusive disease (VOD), post-transplant ileus and a *C,difficile* infection. Ten participants (100%) required antiemetics for nausea, seven participants (70%) required analgesia. Of these, two participants (two probiotics) required a patient-controlled analgesia (PCAS). Four participants (40%, 2 probiotics, 2 placebos) required nasogastric tubes and one participant in the placebo group required total parental nutrition (TPN).

Four participants recorded feedback in the submitted diaries. The feedback related to adhering to the schedule for taking the probiotic/placebo and use of the patient diary. There were generally negative opinions recorded on the taste and volume of probiotic, and the challenges in completing the diary daily, especially when judging the degree of nausea experienced by very small children. No additional research costs (apart from funding for the author of this thesis) were required to set up the MaCROS study. This study received NIHR portfolio funding. This enabled direct access and support from research nurses who work within the department at no extra cost.

4.6 Conclusion

The MaCROS study, a double-blind randomised control feasibility study, was successfully developed, opened and completed on the basis of information reported in this thesis. 10 participants were recruited during a six-month period. Whilst the primary outcome of recruiting 20-40 participants was not achieved, significant barriers were noted, providing guidance for future studies. In conclusion, we propose applying revisions to the MaCROS study protocol and extending the study to reassess findings.

5 Evaluation of the MaCROS study

5.1 Introduction

The MaCROS study evaluated the feasibility of undertaking an RCT to investigate the efficacy of liquid probiotics to prevent or reduce mucositis and infection in children diagnosed with cancers. In general, the research protocol was successfully implemented. The MaCROS study demonstrated that health care professionals and researchers were able to comply with the protocol. The main barriers to compliance with the protocol were (i) identifying and approaching all eligible participants, (ii) adherence to completion of diaries, (iii) recruitment to the face to face interviews and (iv) completion of anonymous questionnaires.

5.2 Summary of the MaCROS study

The MaCROS study demonstrated that it is feasible to undertake a future RCT. Ten participants were recruited into this study, and no unexpected severe adverse events were reported. Children and young people of a range of ages diagnosed with malignant haematological and solid tumours were successfully recruited.

Strengths of the study included ensuring the protocol was developed with patients and families in mind. The probiotic and placebo were delivered in liquid form, ensuring they could be given orally or via nasogastric tubing, as some children struggle to take tablets. Patient information leaflets and diaries were designed to be easy to understand and complete. Participants and their families were able to choose from a paper and web-app version of the diary to address the preferred options for data capture.

Diaries, leaflets and consent forms developed for patients and families were designed for different age groups and were developed in conjunction with patients, families, and other health care professionals. In particular, the ‘chemo’ duck leaflet was positively received by families and health care professionals.

The MaCROS study was awarded portfolio status by the National Institute of Health Research, enabling additional support including access to the department’s research nurses. Symprove kindly donated the probiotic and placebo free of charge and provided a donation for the development of the web-app. Other than the funding of the PhD fellowship, no additional funding was required for this study.

5.3 Learning from the MaCROS study

This section discusses the lessons learned from undertaking the MaCROS study. It identifies (i) specific barriers to achieving particular objectives, (ii) which areas of the protocol would benefit from revision, and (iii) potential strategies to overcome barriers in future studies.

5.3.1 Recruitment

Evaluation of the MaCROS study identified that recruiting a sample of 20-40 participants over a six month period was an ambitious estimate. This sample was calculated on the assumption that two new patients would present to the department every week resulting in 50 potential participants over six months. However, this estimation did not consider that some of these patients would not meet the eligibility criteria, e.g. children under the age of 1, and children who are on chemotherapy regimens unlikely to cause mucositis.

At the start of recruitment to the study, there was a reduction in the presentation of new patients, which is a common phenomenon seen in a rare disease speciality. On average, 2-3 new participants are diagnosed weekly, but in the four weeks after the MaCROS study opened, there were just two new patients who presented with a new diagnosis. The week to week variability in patients who presented during this period impacted the recruitment of new patients. Patients who were already receiving chemotherapy treatment while the MaCROS study was open were also considered. Allowing this enabled identification of 10 potential participants, one of which was not approached due to a language barrier. Three (33%) of these 9 ‘ongoing treatment’ patients were recruited to the MaCROS study. Seven of 30 (23.3%) newly diagnosed eligible participants were recruited.

Studies have reported that 50-70% of children and 30% of young people enrol onto therapeutic clinical trials (114). Recalculating the recruitment target using the 39 eligible participants during the six months it was open, 10-20 participants (approximately 26%-50%) appears to be a more reasonable target. However, even though the MaCROS study did achieve the lower goal of recruiting 10 participants, further barriers have been identified.

5.3.2 Difficulties for health care professionals

Five eligible participants were not approached because either (i) they were not identified at the appropriate time, (ii) there were significant language barriers, or (iii) because health care professionals involved felt it was not appropriate to contact the family.

Recruitment to clinical trials is often reliant on health care professionals who act as 'gatekeepers' by screening patients, and at times obtaining consent on behalf of the research team.

How gatekeepers impact recruitment is poorly understood, though it is believed they can influence the willingness to participate. Newington et al reported that Patients approached by their usual doctor, rather than someone unfamiliar to them, are more likely to participate in a study (115). Gatekeepers may consider multiple factors before deciding to approach an eligible patient such as engagement, the patient's health status and their attitude towards research. Gatekeepers are believed to be more likely to contact a participant if they believe the benefits outweigh the risks of taking part in the study (116). However, there is a lack of research exploring how to overcome the barriers health care professionals (HCPs) face when approaching a cancer patient or their parents/carers.

Other factors that can impact recruitment include how HCPs perceive a family, such as assuming which parents aremore likely to decline participation. Such perceptions come about because of the HCPs' previous interactions with families in a clinical setting. When considering approaching an eligible patient, HCPs worry about the reaction of the child or young person, or their family, and how this might affect the HCP-patient relationship.

In the MaCROS study, some health care professionals felt there were too many competing demands to consider when contacting the parents or guardians of individual eligible patients, and for this reason chose not to approach them. It was noted on a number of occasions that health care professionals felt it was not appropriate to approach a family, because the family were still coming to terms with difficult news or a new diagnosis.

Organisational barriers were also recognised as a barrier for health care professionals to approaching eligible participants and their families. Health care professionals (apart from the PI of the study) were required to identify and contact families during their clinical duties. Dealing with the necessary clinical tasks while discussing the MaCROS study with a family was, at times difficult, mainly when dealing with multiple stressful situations. There were times when the intensity of workload, levels of sickness and critical illness on the ward, and in outpatient settings, resulted in senior medical staff being unable to give patient recruitment the priority it required.

Figure 9 summarises the complex multifactorial barriers which may influence a health care professional's decision to approach an eligible patient or their family about participation in the MaCROS study.

Barriers toa, and facilitators of, recruitment to trials have been examined extensively in the past. A systematic review of 37 studies undertaken by Caldwell et al (117) assessed 4 recruitment strategies, focussing on increasing potential participants' awareness of trials. The review focused on: (i) novel trial designs, (ii) recruiter differences, (iii) provisions of trial information, (iv) and incentives. Meta-analysis suggested that using interactive programmes, videos or educational sessions, and monetary incentives, improved recruitment. Increasing patients understanding of the trial process, but using different methods of randomisation and consent did not. However, whilst thirteen of the thirty-nine studies included in the systematic review focused on cancer conditions, none of these 13 studies included paediatric patients.

A review by McDonald et al (118) reviewed recruitment in RCTs that were funded by the UK Medical Research Council and the Health Technology Assessment Programme between 1994 and 2002. It reported that studies which had simple trial designs, support from trials units, or were drug intervention trials were more likely to be associated with successful recruitment. The MaCROS study had a simple trial design but did not receive support from a trials unit. The review by McDonald et al (118) also stated that using newsletters and posters to inform clinical staff and patients, regular site visits, amending the inclusion criteria/protocols, and delivering presentations and workshops to site staff / appropriate groups were successful strategies to increase recruitment. However, the odds ratios reported were associated with wide confidence intervals (CIs), highlighting the uncertainty of these findings. Furthermore the review did not include any paediatric studies.

The recognised barriers associated with recruitment in RCTs has led to the development of interventions targeting recruitment. A study by Rooshenat et al evaluated the impact of the QuinteT recruitment intervention on 5 RCTs (119). The aim of this intervention was to identify recruitment difficulties and implement actions to address these issues. 4 out of the 5 RCTs encountered recruitment challenges and 3 of these improved following interventions implemented. Four of these RCTs were feasibility studies and two of these included adult participants diagnosed with cancer. Both cancer studies were for surgical interventions and had recruitment periods (12 and 21 months) which were considerably longer than the recruitment period in the MaCROS study (6 months) and involved multiple centres.

A Cochrane systematic review undertaken by Treweek et al reviewed strategies for improving the recruitment of participants to RCTs for a wide range of diseases (120). It identified 68 trials (totalling over 74,000 participants) that implemented recruitment interventions. The interventions focused on trial design, trial conduct changes, modifying the consent processes and information delivery to participants, interventions

aimed at recruiters, and incentives. However, only 2 studies that demonstrated an increase in recruitment were supported by high certainty evidence. The interventions implemented in these 2 studies were (i) using non-blinded rather than blinded placebo trials, and (ii) telephone reminders for people who do not respond to postal enquiries. Using a bespoke participant information leaflet, which is user-tested, was supported by high certainty evidence but demonstrated little to no effect. Treweek et Al suggested focusing on improving evidence based current strategies rather than developing new ones. They suggested using Studies Within a Trial (SWAT) for evaluation of recruitment (121), and contacting Trial Forge (www.trialforge.org) about intended recruitment evaluation, to ensure better coordination and dissemination of intentions.

However, these interventions focused on studies of adult participants, and a paucity of strategies which target paediatric studies was noted. The review by Treweek et al also highlighted the lack of studies targeting interventions at paediatric recruitment and only one study included children (120). Whilst interventions such as the QuinteT recruitment intervention could be applied to paediatric studies, it would have required modification to factor in proxy parental consent, and would have needed analysis by those experienced in clinical trials involving children.

Strategies which could be used to increase recruitment in a future RCT, based on the barriers identified in the MaCROS study, are summarised in table 20. As large-scale recruitment appears to be a significant barrier in many paediatric RCTs, it is clear further research is needed to develop and validate interventions and create a framework which can help increase recruitment in future studies.

Figure 9: Summary of factors which can influence a health care professional decision to approach a patient.

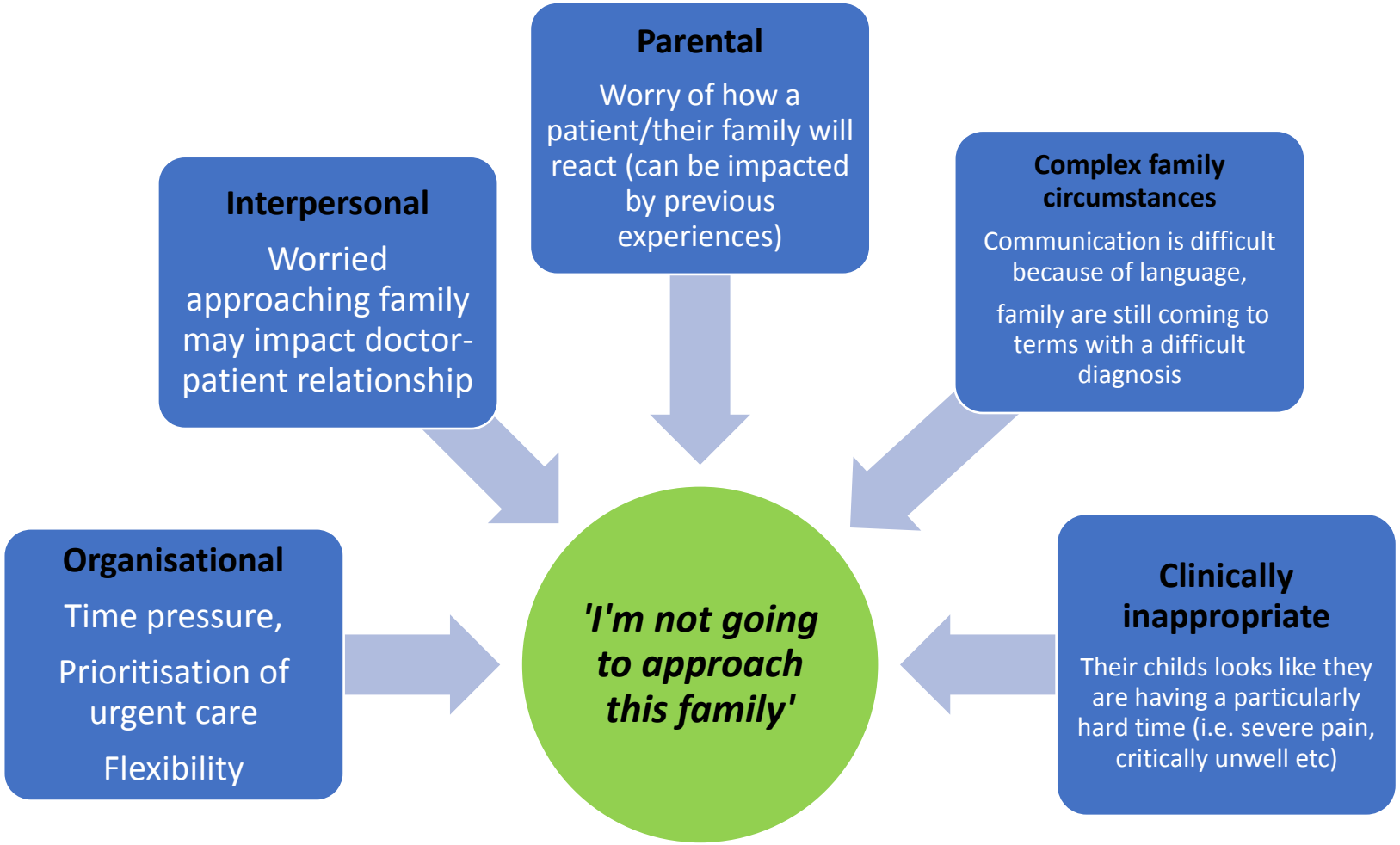


Table 20: Strategies to overcome barriers which impacted recruitment in a future RCT

Barrier	Strategy
Organisational	Revise eligibility criteria – consider greater flexibility on the start date of probiotic/placebo. Extra support- i.e. the initial enquiry is delivered by health care professional known to the family (e.g. named consultant) with research nurse- who can then complete the rest of the consenting process. Predeveloped research packs (including PILS, consent and prescribing forms) to reduce the burden on the health care professional. Increase recruitment period (i.e. 12 months or longer) Use SWAT analysis and consider involving Trial Forge
Interpersonal	Having the named consultant/health care professional who has an established rapport with the family approach initially Approach
Parental	Consider strategies to help support families during stressful situations. Increase the awareness of research from diagnosis, e.g. have a research nurse join the consultant and Macmillan nurse. Having a named research nurse for each family who will approach the family for all potential studies, thereby establishing rapport Have someone who has already established a rapport with the family initially approach (e.g. named consultant).

Barrier	**Strategy**
Not fluent in written and spoken English	Patient information leaflets in commonly used languages (e.g. Urdu) and access to interpreters
Clinical	Enhanced training of health care professionals using presentations, multimedia and group sessions. Consent and randomisation to take place in advance (e.g. completed in cycle two but prescribe at the start of cycle one of chemotherapy)

5.3.3 Barriers impacting patients and families

Whilst many families expressed interest in the MaCROS study, and 10 participants were successfully recruited, some parents chose to decline participation. A breadth of parental opinions and decisions impacted their choice.

At times patients and families chose not to participate in the MaCROS study without reading the patient information leaflets or further discussion. Being diagnosed with cancer as a child or young person is a challenging and life-changing experience. Patients and their families may be experience feelings of overwhelming sadness, hopelessness, anger or despair. Because of this, they may experience difficulty processing their thoughts and feelings, and may not be able to focus on matters which they do not feel are critical for the current situation. In the weeks following a diagnosis, some parents/guardians stated that they felt too overwhelmed and did not want to consider the MaCROS study further. An example of this is a discussion that occurred after the MaCROS study closed with a parent of a child diagnosed with Non-Hodgkin's lymphoma. The following text is paraphrased anonymised:

"When you approached me about the MaCROS study nearer to X's diagnosis, I was devastated and overwhelmed. My child was diagnosed with cancer, could potentially die, and I had to manage this while looking after X's younger siblings and everything else... I said no because I felt I had too much on and couldn't deal with another thing to think about…however, seeing X develop mucositis and how much he suffered. Knowing what I know now, I would have considered it".

This theme has been highlighted in other paediatric cancer trials. Parents of children diagnosed with a life-limiting brain tumour, a diffuse intrinsic brain stem glioma (DIPG), consented to post-mortem biopsies for research purposes. Parents cited that an optional biopsy at diagnosis was a challenging decision to make, but after their child's death, they wanted to help make a difference in the future management of other children diagnosed with DIPG (122).

The role of proxy consent from a parent on behalf of their child may also impact their decision to participate in a clinical trial and this affects recruitment (123). One study reported that parents felt deciding on behalf of their child was harder than deciding to consent for themselves (124). Another study reported how some parents felt that whilst they could accept certain research risks for themselves, they were much less certain about accepting these risks for their child (125). These parents also reported that they would be prepared to take greater risks in treatment for their child for the hope of cure (126).

‘Research fatigue’ was another theme noted. A number of families expressed a desire not to be approached about the MaCROS study because they have already been contacted about other research trials and felt ‘saturated’ by the requests. Patients and families have a fixed amount of available time and resources. They need to use this to manage their illness, health, stress, social and family circles and self-care etc., and this may impact or influence their decision to participate. Kenten et al. (127) explored some of the barriers that occurred in recruiting young people to the observational BRIGHTLIGHT study, which intended to ask all young people newly diagnosed with a malignancy, between 2012 and 2015, to describe their treatment journey and emotional/psychological response. It identified how patients needing treatment felt they should prioritise their time, and that engaging in research was an additional burden. Therefore, strategies to minimise the burden on families should be considered in future studies. Strategies could include focused interventions on relieving the pressure felt by families, by, for example, having a research nurse sit with a family and assist the completion of questionnaires/diaries etc.

Recruitment may have also been impacted by the relationship patients and their family have with the health care professional who first enquired about participation in the MaCROS study. Studies have previously reported how families may be more likely to participate in a trial when approached by a professional they have already established rapport and confidence with (128-130). In the MaCROS study, a number of professionals initially approached patients and families. This included named consultants, the PI and PhD fellow (HH - who was a paediatric registrar within the department) and research nurses. HH worked within the department at weekends on an ad-hoc basis as a junior member of the clinical team and was present on the wards and clinics with other clinicians. Whilst HH established rapport with some families; at other times HH had to approach families without previous contact. This may have impacted negatively upon recruitment. A strategy to overcome this in future studies is having the patients’ named consultant approach the patient or family about participation. An alternative strategy includes changing the way research nurses are allocated to trials. Currently, at LTHT haematology and oncology department, research nurses are typically designated responsibility for a particular study. Therefore, a family may be approached by various research nurses about different trials. The relationship between research nurses and families could be strengthened by allocating a specific research nurse to the family. Allocating one research nurse to the same family could help increase the success of establishing rapport. However, this strategy would need further consideration of the impact on practicalities and cost.

Patients or parents may also be more likely to engage with a discussion about a clinical trial if they feel it has value, i.e. a patient may be more likely to consider a trial which has potential curative intent. Alternatively, they may be more likely to engage with a clinical trial if it requires less ‘effort’. For example, patients and families may agree to participate in a study which involves taking a blood sample for research purposes alongside other necessary routine interventions. They may prefer not to participate in an interventional trial or qualitative studies, since these studies may or may not improve symptoms. Patients and families may feel that certain studies are more ‘burdensome’ and therefore, a lower priority.

Barriers that also appeared to impact a patient/their parents' decision to participate in the MaCROS study included the possibility of receiving a placebo or having to complete a patient diary. Some patients/parents disliked the option of receiving the placebo and felt the 'risk' was not worth it. Others stated they were interested in receiving the probiotic/placebo but were put off by having to complete a diary every day for 21 days. Some of these barriers are demonstrated in figure 10.

A paraphrased statement which was mentioned a few times when declining to participate was:

‘*I don’t want to do it if there is a chance I will be in the placebo group'.*

Similar barriers have been previously explored in studies. A systematic review by Beasant et al (131), which included paediatric cancer studies, reported how parents may have a treatment preference which could impact participation in a randomised controlled trial. Beasant et al discuss the use of patient preference and comprehensive cohort trials - in which participants with a preference are offered their treatment of choice, and those without are randomly allocated treatment. However, the use of non-randomised studies reduces the robustness of their findings. An alternative option includes undertaking a cross-over clinical trial to ensure participants receive both the probiotic and placebo. However, it is not clear what the impact of chemotherapy and antibiotics on probiotics would be in a cross-over trial.

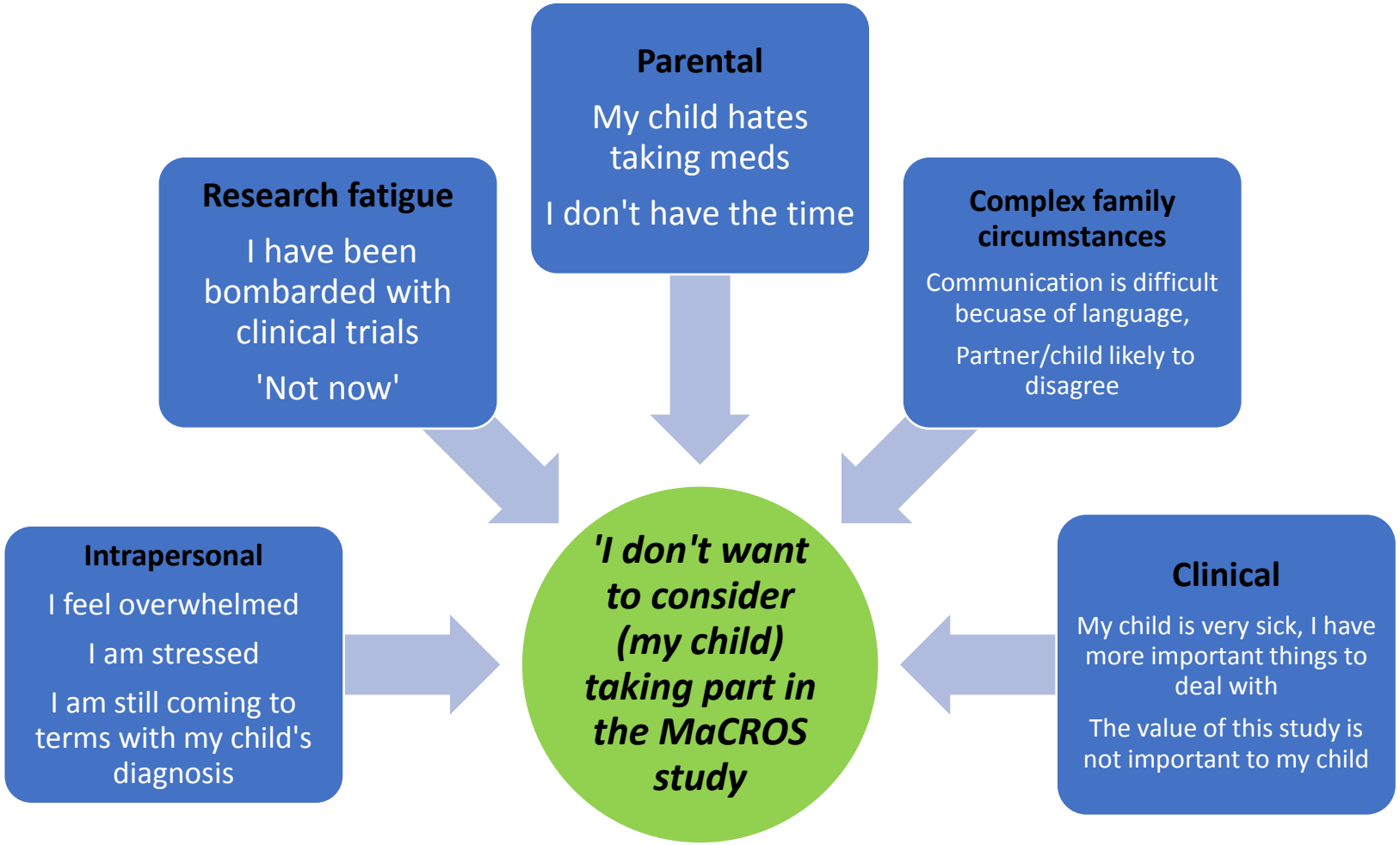
Whilst studies have identified and explored factors which influenced parents’ choices in taking part in clinical trials, a lack of studies which focused on interventions to overcome barriers to parent participation was noted. Further consideration of strategies targeting paediatric trials may help increase recruitment trials which have similar issues to those identified in the MaCROS study.

5.3.4 Other barriers that impacted recruitment

Children and young people who were eligible for the MaCROS study may have also been eligible for other studies. As a consequence, trials may be in 'competition' with each other for the consent of a participant. A review of the literature identified research exploring competing clinical trials as a theme (132), highlighting how recruitment competition can impact studies. Exploration of competing clinical trials focusses on studies targeting specific patient populations and studies with similar outcomes, where the patient can enrol in only one of the studies. An example of such studies are phase 1 studies exploring novel interventions for curative intent for children with relapsed high-risk neuroblastoma. There is a lack of research exploring competition between different types of trials (e.g. interventional vs observational), and what patients' and parents' views around this are.

It is also worth mentioning that the MaCROS study was classified as a non-CTIMP study by the MHRA. However, the MHRA stated that recommendation would have to be reviewed before the undertaking of any future research. Being classified as a CTIMP study may have a profound impact on recruitment as participants are currently forbidden from enrolling from more than one CTIMP trial at a time. Many patients who meet the eligibility criteria would, therefore, be excluded because they are on another larger, protocol driven CTIMP trial. This is something that would have to be considered or reviewed in future studies.

Figure 10: Summary of factors influencing a patient or their families decision to participate in the MaCROS study.



5.3.5 Barriers to patient diary completion

Two major issues were identified in relation to the patient/symptom diaries. These were

- Completion of the full information over the course of the 21 days
- Return of the diaries

Even though the patient diary was created to minimise the amount of effort and time needed to complete it, we were not able to achieve the intended outcome. This was despite giving participants and their parents the choice of using either a web-app or paper diary. Some parents expressed an interest in using the web-app, but stated that the poor WiFi connection in the hospital dissuaded them from this. Therefore, a poor WiFi connection was identified as an organisational/system barrier which impacted how participants chose to collect data.

The proportion of each diary completed appeared to decline at multiple points during the study; some participants did not return a diary or had only completed data for one or two days of the study. This may be because they had already received the probiotic/placebo, and completion of the diary was therefore viewed as a task which had little direct benefit to themselves. The diaries may have been considered to be burdensome - juggling complex family circumstances, an ill child and additional stress making it difficult to remember to complete the diary. A participant may also have been interrupted when completing the diary affecting their 'flow' and concentration or cause them to miss questions.

Attrition in completion of the diary occurred a further time, at day 14, when participants had completed the full course of probiotic/placebo. Only three participants completed any information beyond this point, and none completed information on all 21 days. We suspect this attrition occurred because the participants had finished their course of probiotic/placebo, and they had fewer incentives/reminders to complete the diary.

As completing a diary was a time-consuming requirement of the study, strategies to reduce the burden on participants/and their families should be considered in future studies. A study by Okupa et al (133) explored the impact of the patient burden by comparing the use of daily diaries with retrospective questionnaires, for children and young people with asthma. The study reported that daily symptom diaries increased reporting of symptoms from the most recent two weeks, when compared with retrospective questionnaires. It suggested that diaries should not be used interchangeably with retrospective questionnaires and that the nature of the hypothesis should indicate what should be applied. Studies which require greater accuracy may

benefit from the use of diaries. It is worth noting Okupa et al (133) stated that the use of electronic web diaries might increase adherence. While there were a small number of MaCROS participants who chose to use the web-app, 3/3 returned a partially completed diary.

The robustness of data vs. completeness of data need to be considered before further research, based on MaCROS, is undertaken. Future versions of the MaCROS study may benefit from an increased drive to use web-app diaries with a daily reminder. An alternative option could include a research professional (e.g. research nurse) telephoning participants every week during the three weeks, and completing a standardised form over the phone, regarding symptoms during the previous week. However, this is likely to result in recall bias, and as previously highlighted may not be beneficial in studies which are investigating outcomes requiring greater accuracy.

Gifting or rewarding participants for returning diaries/taking part in questionnaires is also an option. The ethical implications of this have been extensively explored in previous studies (126, 134, 135) and the HRA have issued guidance on payments and incentives in research which states that this is acceptable, in certain circumstances, for adults (136). This HRA guidance also references the Medicines for Human Use (Clinical Trials) Regulations (2004) for payments to children in CTIMP trials, stating that financial incentives cannot be given for paediatric CTIMP trials. The Royal College of Paediatrics and Child Health have issued guidance for non-CTIMP studies (137). It reports that financial inducement should not be offered but that expenses should be paid. Expenses could be given to future participants for the time required to complete the patient diaries by asking them to complete an invoice. Participants can also be given pre-stamped envelopes to return diaries, daily reminders via text or email to complete information for both paper diaries and web-apps, or gifts/donations for returning diaries. This could include the gifting of vouchers or a monetary fee of reasonable value for the time estimated to complete the information.

5.3.6 Barriers to study-procedure interviews and questionnaires

The MaCROS study was initially designed to use ‘declining participant’ questionnaires and interviews with those who had completed the study. No participants opted to give feedback using interviews; no participants returned anonymous questionnaires or participated in the interview. Reasons for this appear to be the effort required/time commitment for participants and families while 'juggling' other requirements such as (i) family commitments, (ii) attending patient clinics, (iii) dealing with unexpected changes,

and (iv) unplanned additions to the treatment/care. However, because the MaCROS study was an underpowered study, these findings could have occurred by chance.

Should further studies based on the MaCROS study wish to investigate the reasons for non-participation and how to improve the practical implementation of the protocol, it will be necessary to look at removing the burden of a time-intensive feedback process. For example, rather than ask patients, or their parents or guardians, to fill in an anonymous questionnaire explaining why they declined to participate, the researcher/responsible clinician could complete a proforma following a verbal discussion. This part of the feasibility study is not necessarily required in a future study and could be removed from the protocol, or used in a limited phase early on, as part of a QuinteT-style approach. Participants/families could be offered a range-methods to deliver feedback, i.e. returning feedback using a prepaid envelope, completing feedback online, or requesting a meeting with a research assistant who can help them provide feedback.

5.3.7 Completion rates of probiotic and placebo

Eight participants (3 probiotics, 5 placebos) out of ten completed the full course of intervention. Two participants dropped out of the study after the first dose because they did not like the taste of the liquid (one from each arm). One participant had to omit several doses due to being nil by mouth. The MaCROS study demonstrated that even though a majority of participants completed the course of probiotic/ placebo, taste and mode of delivery can impact adherence. Modifications to the frequency, dose, and taste of the probiotic/ placebo may therefore improve compliance. Increasing the frequency of dosing would reduce the volume required for each dose. On the other hand, the increased burden of delivery could be an issue for some participants or their parents/caregivers.

In an attempt to minimise the barrier to participant adherence and retention presented by poor taste, methods to mask the taste were explored. A healthy family (two parents, and two children aged 3 and 7) were asked to drink the Symprove probiotic in different combinations. The Symprove probiotic was added to a variety of drinks, including Lucozade, lemonade, sugar-free cordial (Robinsons), orange juice, and milkshake. The parents and their children stated that the probiotic tasted best in the sugar-free cordial. Therefore, for the remainder of the study participants were advised to mix the probiotic/ placebo with sugar-free cordial juice if they could not tolerate the taste. However, because the taste was only explored in a healthy family, it is unclear how chemotherapy could impact the taste experience by cancer patients receiving the probiotic. In undertaking a larger study, the palatability of probiotics must be thoroughly explored.

5.3.8 Preliminary health economic information

Participation in the MaCROS study did not impact patient care. As recruitment, consent and randomisation occurred when participants were already in the hospital for routine patient care. There were no reported unexpected serious adverse events attributed to the probiotic or placebo. Patients' hospital stays were not impacted, nor were supportive care strategies required.

A cost/benefit analysis was submitted as part of the application to the HRA. No additional research costs (apart from funding for the author of this thesis) were required to set up the MaCROS study, and this study received NIHR portfolio funding. This enabled direct access and support from research nurses who work within the department at no extra cost. A research nurse communicated with participants, approached eligible participants to join the study, supplied PILS, and collected diaries from participants upon completion. The company Symprove provided the probiotic and placebo to the MaCROS study free of charge, and donated costs to the development of the web-app. Evaluation of the study demonstrated that participation did not contribute to any additional clinical care, or incur any further cost. However, any future study would require external funding for it to continue, in particular if a multi-centre RCT is undertaken, since that would require more clinical and research staff as well as input from a clinical trials unit.

5.4 Other considerations

5.4.1 The microbiome

Research has recently explored how the state of the microbiome before, during and following a cancer diagnosis can be linked to the development of toxicity (i.e. infection) and response to interventions (75). This dysbiosis (the disruption of the microbiome) can affect the health of an individual and may vary at different stages of a cancer patient's journey, i.e. before diagnosis, during treatment, and at the end of treatment. Overall, there is a lack of research to understand the role of dysbiosis, particularly in children.

As previously discussed in chapter one, the gut microbiome may affect drug metabolism and efficacy, and how an individual responds to chemotherapy (62). The gut microbiome is impacted during cancer treatment by a range of factors, including (i) diet, (ii) surgical intervention, (iii) supportive care interventions, i.e. antibiotics, and (iv) chemotherapy. Research by Panebianco et al (69) proposed that in mice bacterial translocation and T-helper 17 cell activation increases the efficacy of

cyclophosphamide in a 'healthy' gut microbiome, when compared to mice raised in a sterile environment. Mice with a reduction of gram-positive bacteria, due to vancomycin, were found to have a reduced therapeutic response to cyclophosphamide, when compared to the control group. *Lactobacillus johnsonii*, *Lactobacillus murinus*, and *Enterococcus hirae* were found to stimulate an Th1 and Th17 immune response in the spleen and mesenteric lymph nodes, thereby improving the effectiveness of cyclophosphamide. The study authors Viaud et al (70) were unable to demonstrate this response in mice that received vancomycin.

However, although this has been demonstrated in murine studies, the impact of intravenous antibiotics on the gut microbiome and how this affects the therapeutic response to cyclophosphamide in humans has not been investigated.

A further recent study by Zieglar et al 2019 (72) evaluated the impact on the gut microbiome of a prophylactic antibiotic (levofloxacin) (prescribed to prevent febrile neutropenia). The study compared this to broad-spectrum beta-lactam (BSBL) antibiotics to treat episodes of fever. In both groups (totalling 60 patients) the patients had haematological malignancies (72). It was found that the gut microbiome of patients with BSBL exposure had significantly reduced diversity when compared to those without. This study proposes that the gut microbiome may interact with the delivery of chemotherapy and supportive care interventions, potentially impacting the toxicity experienced by a patient in their current and future courses of chemotherapy (72). This research stands alongside the work suggesting probiotics, delivered during a course of antibiotics, may reduce antibiotic-associated diarrhoea (43), and emphasises that how antibiotics and probiotics interact remains uncertain.

There is still a lack of research which has fully explored the pharmacology, pharmacokinetics and pharmacodynamics of probiotics, particularly in immunocompromised patients and children. This sparseness of data meant it was not possible to use robust scientific evidence to justify the commencement and duration of the course of probiotics in the MaCROS study. Through considerations of the practical delivery of the intervention, for the study we chose to supply the probiotic over a fixed period of fourteen days, rather than throughout treatment. Assessment of the microbiome, gut flora and bacterial colonisation could identify advantages or disadvantages of probiotic use and mucositis. It would enable understanding of the mechanism relating the bacterial composition of stool samples to outcomes. Further exploration of how probiotics may impact upon bacterial diversity could help guide how to dose probiotics, and for how long, in future clinical trials.

5.4.2 Biological sub-study

Once the MaCROS study had opened, the need to research the biological component of using probiotics was identified. Therefore, a parallel biological feasibility sub-study was developed to be undertaken in conjunction with the MaCROS study. This sub-study aimed to investigate the mechanism of action (or lack thereof) of probiotics in children with febrile mucositis, by investigating the presence of a biomarker (faecal calprotectin) and bacterial diversity in stool samples.

Biomarkers are defined as “human or animal biological property whose in vitro measurement or identification is useful for the prevention, diagnosis, prognosis, treatment and follow-up of humans or animal diseases, and for their understanding (138)”.

A review of the literature highlighted that biomarkers have been used to identify or stratify the risk of mucositis in adults (139, 140) .Ten biomarkers have been investigated in 4 paediatric studies (141) (142) (143) (144). These studies reported that:

- Serum citrulline may be used to determine the severity of mucositis (143)
- Faecal calprotectin may be used as a non-invasive biomarker for those with mucositis without neutropenia (141)
- Serum procalcitonin may be able to distinguish fevers due to bacteria from those with mucositis who are febrile due to a systemic inflammatory response (144)
- The C-Sucrose breath test is feasible to use in children with cancer (142)

Whilst serum IL-8 is a potential biomarker in children with febrile neutropenia it may not be accurate for use in those who also have mucositis.

The four trials were reported as prospective studies by research authors (including one randomised-control trial), however two of these studies reported on a subgroup of participants, of which samples were analysed retrospectively.

All studies had small sample sizes and reporting of the studies was unclear. Significant biases were found in these studies, including selection bias (142) confounding bias (all studies) and outcome information bias (all studies). Reporting of statistical results did not include 95% confidence intervals.

The studies used different definitions and grading tools for mucositis. Gosselin KB et al (145) and Tooley KL (142) on oral mucositis only, WJFM van der Velden et al (143) included those with oral and gastrointestinal mucositis (using different grading tools), whilst KG Miedema et al (144) did not make any reference to oral mucositis. It is unclear from these studies whether oral, gastrointestinal or combined mucositis would impact the interpretation of biomarkers.

This review highlighted the need for further robust studies to explore how biomarkers can be used to investigate the response probiotics may have on mucositis. Faecal calprotectin is a non-invasive biomarker with a relatively low cost to undertake analysis. One stool sample can be used to simultaneously analyze bacterial composition and biomarkers. This is less invasive and more convenient for participants then requiring to attend hospital for a blood test.

Therefore, an amendment to the MaCROS study to include a biological sub-study was proposed. This would investigate the feasibility of testing stool samples for probiotic bacterial colonisation, and faecal calprotectin as a biomarker to explore the effect of probiotic consumption in children with mucositis.

However, it was not possible to pursue the sub-study while the MaCROS study was open, due to challenges in submitting an amendment to the HRA, seeking permission from the LTHT REC and LTHT R&D, and setting up the trial with the local microbiology department,. The biological sub-study protocol developed is enclosed in appendix 2.19 , and it is proposed that future versions of the MaCROS trial should include a parallel biological sub-study investigating the mechanism of probiotic response.

5.4.3 Alternative strategies

A number of options can be considered to improve on the limitations of incomplete data and recruitment that were identified in the feasibility study. These include:

- Undertaking an RCT with patient public involvement to enhance recruitment and compliance (previously discussed in this chapter).
- Undertaking a further feasibility study prior to undertaking an adequately powered RCT
- Undertaking a pilot study which can be embedded into an RCT

5.4.3.1 Undertaking a further feasibility study

Undertaking a second feasibility study, prior to a RCT, would allow further evaluation of changes made to the study protocol with the aim of improving recruitment and adherence to data collection. This could reduce problems that still may occur in a future RCT. A future RCT may still be problematic despite this research identifying, and proposing solutions to, barriers to an RCT.

Demonstrating how the proposed revisions to the protocol have resulted in an increase in recruitment and adherence to the capture of data would help researchers limit inefficiencies in a RCT, and increase confidence in researchers and health funders who invest time and funding into the trial.

However, there are also disadvantages to undertaking a further feasibility study, such as dedicating more time, effort, and costs to evaluating the study design and protocol. Table 14 demonstrated that 4 out of 6 objectives evaluated did not need further modification; undertaking a further feasibility study would result in unnecessary repetition of parts of the research protocol..

5.4.3.2 Undertaking a pilot study that is embedded in an RCT

Undertaking a pilot study (a ‘preliminary study’) that is embedded in a future RCT is also an alternative option to undertaking a full RCT. Like a feasibility study, a pilot study can guide the design of a study, while allowing the opportunity for modifications to the protocol to take place prior to completion of the study. Whilst a feasibility study also evaluates the implementation of the protocol, its data cannot be used in an adequately

powered RCT. Therefore, data from a feasibility study cannot be used to draw conclusions regarding efficacy. However, data from a pilot study can be included in the intended RCT whilst giving the opportunity to evaluate study design.

Advantages of a pilot study over a further feasibility study are (i) a reduction of costs, and (ii) avoiding unnecessary repetition and time wastage. It would enable further evaluation of the revisions made to improve recruitment and data capture adherence, these improvements having been identified from the feasibility study. A pilot study would identify whether the proposed changes appear successful or require further revision. However, a pilot study would not guarantee success for an adequately powered randomised controlled trial. This risks loss of time, funding and effort. The impact of failure could be limited by instead undertaking a further RCT.

6 Conclusion

6.1 Introduction

This thesis has reported that the survival rate of children diagnosed with cancer has increased during recent decades, because of earlier diagnosis, and improved curative treatment and supportive care strategies. However, death attributed to treatment-related mortality, particularly infection-related mortality from increased toxicity of intensive treatment strategies, remains a significant concern.

This thesis identified how treatment-related mortality (TRM) is poorly defined and reported in studies. It then demonstrated how increasing the accuracy when reporting TRM vs infection-related mortality (IRM) would harmonise results. It was in turn demonstrated this would enable better comparisons of clinical trials, with knowledge gained applied to understanding the role of IRM in TRM.

This thesis then demonstrated the usefulness of a validated classification tool and cause of death attribution system, which could guide future research and supportive care strategies. The thesis also highlighted the impact of infection and mucositis on treatment-related morbidity and mortality.

From this, two critical areas of research were identified and proposed that required further exploration:

1) The need for a uniform definition of TRM and disease-progression for the classification of death in children with cancer, applicable in high-income countries
2) The need for preventative and treatment strategies to reduce TRM in children diagnosed with cancers, potentially through the modification of the microbiome to reduce mucositis, bacterial translocation, and bloodstream infection

This led to the review of preventative supportive care strategies, which could potentially reduce the burden of mucositis and infection, and introduced the concept of using probiotics in people and children with cancers. A paucity of evidence was highlighted, and used to demonstrate that further research was required.

6.2 Aims and data collected

Based on these findings, the following research was undertaken to explore this in further detail:

- A newly developed definition of TRM using a classification tool and cause of death attribution system was validated in a study undertaken at Leeds Teaching Hospital Trust, UK
- An updated systematic review and meta-analysis was undertaken to explore the efficacy and safety of probiotics in people with cancer
- A double-blind randomised control feasibility study was undertaken to investigate the feasibility of an RCT investigating the use of probiotics to prevent or reduce mucositis and infection in children with cancer

A classification tool and cause of death attribution system was validated at LTHT, a hospital outside the country where it had been developed. Thirty medical records of the most recent deaths in children with cancer, 2 and 4 weeks prior to death, were anonymised and presented to the participants. Reviewers independently classified deaths as ‘treatment-related mortality’ or ‘not treatment-related’ according to the algorithm developed. When TRM occurred, reviewers applied the cause-of-death attribution system to identify the primary cause of death. Inter-relater reliability was assessed using the kappa statistic (k).

Reliability of the classification was deemed ‘very good’ between CRA and consultants (k=0.86, 95% CI 0.72 to 0.97). Ten deaths were classified as TRM, of which infection was the most frequent cause identified. Reviewers disagreed on the primary cause of death (e.g., respiratory vs infection), in six cases, when applying the cause-of-death attribution system, and disagreed on the probable and possible causes in four cases. Out of the 10 patients who died from deaths due to TRM, 6 were attributed to infection. The study identified how the algorithm might not detect TRM in patients receiving non-curative therapy.

The findings led to the conclusion that further preventative strategies to reduce the burden of infection-reduced morbidity and mortality were required.

A systematic review and meta-analysis were undertaken to explore the use of probiotics to reduce or prevent symptoms of mucositis and infection in people with

cancer. Randomised control trials (RCTs), identified through screening multiple databases, were included for analysis of efficacy. Non-randomised control trials and case reports were included for safety analysis. Outcomes included a reduction in the incidence and severity of diarrhoea and adverse events. Where possible, data were combined for meta-analysis using a random-effects model. Planned subgroup analyses were not possible due to marked heterogeneity of study characteristics.

The systematic review demonstrated that probiotics appear safe to use in this group of patients, and may reduce the incidence of diarrhoea and duration of fever, but that there is uncertainty regarding the risk of bias from included the studies (table 8, figure 4), and therefore a lack of confidence in the studies' conclusions. The review also highlighted a significant lack of paediatric studies investigating the use of probiotics in this group of patients. Twenty-one studies (N = 2982 participants) were included for assessment of efficacy. Probiotics may reduce the incidence of diarrhoea in patients with cancer [odds ratio (OR) = 0.52, 95% confidence interval (CI) 0.34-0.78, 95% prediction interval (PI) 0.3-0.92, I-sq 36.9%, 5 studies] and the duration of pyrexia [standardised mean difference 0.39 days, 95% CI 0.35-0.43, I-sq 0.01%, 5 studies]. The systematic review demonstrated that there are insufficient studies to assess the true effect of probiotics in people with cancer. Meta-analysis suggested probiotics may be beneficial, but further studies were required, particularly in children.

Twenty-five studies (N = 2242) were included in the safety analysis. Five case reports showed probiotic-related bacteraemia/fungaemia/positive blood cultures. Definitions and reporting of adverse events were variable and inconsistent. It was identified that improved reporting of outcomes and adverse events in clinical trials are required to improve the accuracy of future studies and confidence in the conclusions drawn from them.

This provided the rationale for developing a randomised control feasibility study, before undertaking an RCT, to investigate the efficacy of probiotics for reducing and preventing infection and mucositis in children with cancer. The MaCROS study recruited 10 participants between May and November 2019, from 34 who were approached and 39 who had been deemed eligible.

Four participants were randomised to receive the probiotic and 6 participants received the placebo. Of these, 8 participants (3 probiotics, 5 placebo) completed the full course. Seven participants returned partially completed diaries (4 paper diaries, 3 web-app).No

participants completed data for all 21 days. Three of the seven participants completed 80% of the data for the duration of the 14-day course of probiotic. There were no deaths, and no participants were admitted to intensive care. Expected serious adverse events which occurred included one participant developing neutropenic enterocolitis, vaso-occlusive disease (VOD), post-transplant ileus and a *C. difficile* infection. Ten out of ten participants required antiemetics for nausea, 7/10 participants required analgesia. Of these, two participants in the probiotic arm required a patient-controlled analgesia system (PCAS) Four participants (2 probiotics, 2 placebos) required nasogastric tubes and one participant in the placebo group required total parental nutrition (TPN).

No patient who declined consent opted to fill in the anonymous feedback questionnaire although verbal feedback was given at the time. No participants agreed to an interview regarding the study, but four participants gave feedback in the diaries supplied. Feedback received from participants and family was generally positive, and many families expressed interest in taking part in the MaCROS study. Those who received and reviewed the participant information leaflets stated that the leaflets were easy to read and understand, and that they covered all necessary information. The ‘chemo duck’ leaflet was particularly appreciated by children and families.

Feedback documented in symptom diaries, from participants or their parents/guardians, identified aspects of the MaCROS study that require further consideration. These included (i) reviewing the taste and volume of probiotic delivered, (ii) improving adherence to the capture of data, and (iii) assessing symptoms in children who are unable to communicate their experiences.

The MaCROS study demonstrated that an RCT is feasible, and no unexpected serious adverse events were reported. Modifications to the protocol will need to focus on recruitment strategies and adherence to data capture. More support for participants and their families to complete diaries is essential.

6.3 Impact on children diagnosed with cancers

6.3.1 Classification of death

The findings from the TRM study support the hypothesis that the classification system can be implemented effectively in different health care settings, thereby improving the consistency and accuracy of outcome reporting in trials and clinical practice. The classification tool and cause of death attribution system can be used to guide local practice, and prompt guideline reviews to further improve supportive care interventions.

Whilst these findings would not directly impact children diagnosed with cancers, implementing the classification tool and cause of death attribution system could improve the care for children subsequently diagnosed. Regular auditing of deaths using the classification tool and attribution system could consistently identify the cause of death within a department. Such findings can then be used to review local guidance or implement quality improvement projects. For example, an audit of TRM attributed to bleeding could prompt a review of platelet transfusions by increasing or decreasing the threshold for undertaking a platelet transfusion. Increasing the threshold for platelet transfusions, because a higher number of deaths are attributed to bleeding, could reduce the occurrence of spontaneous bleeds. Similarly lowering platelet thresholds, because an audit demonstrated a low incidence of TRM deaths attributed to bleeding, could result in the reduction of unnecessary delivery of blood products.

6.3.2 Probiotics

The systematic review undertaken to investigate the use of probiotics in people with cancer established that there is still insufficient evidence to conclude that probiotics are effective and safe in people with cancer. The meta-analysis demonstrated that probiotics might reduce (i) the incidence of diarrhoea, (ii) duration of pyrexia, and(iii) incidence of septicaemia and central line infection. It was not possible to perform subgroup analysis, particularly in children, to investigate this further. Probiotics may be a rare source of infection, but no deaths have been attributed to their consumption.

Findings from the systematic review and meta-analysis could prompt local centres to review neutropenic guidelines, and allow children diagnosed with cancers to take probiotics. Whilst there is not sufficient scientific evidence to demonstrate the benefits of probiotics in children with cancers, it would give participants and their families the autonomy to make an informed decision about taking probiotics.

6.3.2.1 Participation in feasibility studies

The MaCROS study has identified the need for further strategies to improve recruitment, adherence to the probiotic dosing, and the adherence for completion of data. These findings can be used to improve the experiences of children and their families who participate in future RCTs. Findings can also be used to improve the participants experience in other feasibility studies - particularly those investigating the use of probiotics or studies which require capture of proxy or self-reported symptoms.

6.4 Implications for national recommendations and guidelines

6.4.1 Classification of Death

The classification of death and cause of death attribution system can be used to guide national policies and agendas. This could include developing recommendations or identifying research needed to improve supportive care. Findings could be used to research curative strategies.

6.4.2 Probiotics

The findings from this systematic review could be used to support recommendations in adult guidelines. The Multinational Association of Supportive Care in Cancer (MASCC) 2014 issued guidelines on the management of mucositis secondary to cancer therapy. This guideline advises that probiotics containing *Lactobacillus* species should be used to prevent diarrhoea in patients receiving chemotherapy and/or radiation therapy for a pelvic malignancy but has not referenced the evidence for this recommendation.

In 2018 the European Society for Medical Oncology (EMSO) issued guidelines for the management of diarrhoea in adult patients with cancer (146), including the use of probiotics. The guideline discusses the conflicting evidence reviewed for the use of probiotics (grade II B) and mentions how probiotics may reduce the incidence of diarrhoea, but that there is a higher risk of developing a severe infection in immunocompromised patients. The review undertaken for this PhD has demonstrated that probiotics may be a rare source of infection but that no deaths have been attributed to their consumption. The guideline could be revised based on the findings of this study.

In the UK, neutropenic diet guidelines for haematology patients state that patients should avoid consuming foods or supplements with probiotic cultures. It states that

Lactobacilli and Bifidobacteria do not have a more significant infection risk than strains found in the mouth, ileum and colon, and that reported infections are mostly limited to *Saccharomyces boulardii/ cerevisiae*, *B Subtilis* and *Lactobacillus rhamnosus LGG*. Findings from the neutropenic diet guidelines appear to be in keeping with our updated systematic review, although evidence to support this is limited.

6.5 Limitations of this PhD

The TRM study reported that although consultants were considered the gold-standard for classifying death, even experienced clinicians can disagree on the cause of death when using the algorithm. The differences identified highlight how the TRM classification tool would never have perfect agreement between reviewers, irrespective of experience, or clinical and scientific knowledge. Whilst the current TRM classification tool can be applied reliably, there still may be confusion as to what the definitions of treatment-related mortality and cancer-related death encompass. Suicide or unrelated accidents/illnesses lead to a lack of clarity in decisions.

The study asked reviewers to attribute death to one primary probable, or possible, cause. Reviewers found it challenging to identify one cause of death, and to distinguish between probable and possible causes, indicating further consideration for future applicability.

The systematic review reported how most domains at risk of bias were reported as unclear due to limited reporting of methods undertaken (table 8). The highest risk was identified when assessing selection and detection bias. Whilst aspects of these biases may not be relevant, e.g. whether participants were blinded to the episodes of diarrhoea, most studies did not report sufficient information about methods undertaken, e.g. whether personnel were blinded from allocation or randomisation. This may have undermined the randomisation process, resulting in biased and inflated effect estimates. It was not possible to perform subgroup analysis due to the high clinical and statistical heterogeneity. Studies had a variety of different strains, doses and duration of probiotics and reported different outcomes. The significant heterogeneity reported indicated uncertainty in the findings reported.

The Loke method for the quality assessment of safety of probiotics identified that studies are still unclear on definitions, measures, and reporting of adverse events. Currently, no consistent definitions are used in the reporting of adverse events and

other outcomes. It was unclear how many individuals sustained adverse events as reporting varied between studies. Some studies reported on individual events rather than people sustaining an adverse event, and it is unclear how this may overlap. For example, some studies reported on the incidence of septicaemia, incidence of pneumonia and UTIs. This made it challenging to identify the number of individuals, or indeed if the same episode of illness was counted in two categories. Some studies did not report on bacterial isolates from positive blood cultures identified in either the probiotic or control groups. Therefore, it cannot be concluded with confidence that there were no probiotic-associated infections, or that adverse events sustained cannot be attributed to probiotics consumed, due to the heterogeneity of malignancies and treatment regimens. As adverse events were also not clearly or uniformly defined in identified studies, it cannot be determined if all relevant data were appropriately identified, recorded or documented.

Results from the MaCROS study was used to evaluate the future feasibility of an RCT and provide descriptive results. However, evaluation of the MaCROS study was impacted because of barriers to recruitment and adherence to data collection. Further consideration of how to improve issues of eligibility, recruitment, retention, and missing data is required for future studies. Overcoming these barriers would increase the accuracy and quality of data reported, therefore increasing the accuracy of studies and confidence in the conclusions drawn.

A number of strategies could be applied to improve issues of eligibility, local departmental effects and recruitment. These include revising eligibility criteria, for instance having greater flexibility on the start date of probiotic/placebo. Using predeveloped research packs (including PILS, consent and prescribing forms) to reduce the burden on the health care professional, increasing the recruitment period (i.e. 12 months or longer), using a SWAT analysis, and involving Trial Forge can improve issues identified. Further supportive strategies can also be applied to aid recruitment and retention. These include delivering extra support through familiarity, such as having the initial enquiry be delivered by a health care professional known to the family. This could be a named consultant or a research nurse, and this person can then complete the rest of the consenting process.

Strategies to improve retention and missing data include further exploring the use of web-app diaries (with daily reminders) and using health care professionals (e.g.

research nurse) to undertake some data collection. Another suggestion is telephoning participants every week during the three weeks and completing a standardised form over the phone regarding symptoms during the previous week. A further suggestion is paying expenses for the time taken to complete the diaries.

Barriers that were identified could be reviewed prior to undertaking a RCT by involving PPI groups and engaging in further evaluation, or further research to address these issues. Options include revising the protocol prior to an RCT, undertaking a further feasibility study, or including changes in a pilot study that is embedded in an adequately powered study.

A lack of research exploring the microbiome and dysbiosis in different aspects of a child's cancer diagnosis was also identified. The unknown factors include how quickly the probiotic takes effect, and the relationship of the gastrointestinal microbiome with cancer, probiotics, and antibiotics in the paediatric population. Better understanding of these factors and how they interact would help guide the dosing and delivery of probiotics and help refine methodologies of future studies.

An absence of research investigating the biological evidence for the use of probiotics in people with cancer was also found. The previously discussed systematic review and meta-analysis did not include a review of the bacterial compositions of the stool samples of included studies, because of the lack of data reported. Investigating the bacterial composition of stool samples would allow further exploration of how probiotics may impact bacterial diversity, particularly in patients who are also receiving intravenous antibiotics.

During the undertaking of the MaCROS study, we identified this need to research the biological component of using probiotics and fully developed a parallel feasibility biological sub-study to investigate the mechanism of action (or lack of) of probiotics in children with febrile mucositis. However, due to the challenges noted in section x.x it was not possible to set up the biological sub-study.

6.6 What was known prior to this thesis?

The survival rate of children diagnosed with cancer has improved during recent decades. Earlier diagnoses, as well as improved curative treatment and supportive

care strategies, have reduced overall mortality. However, death attributed to treatment-related mortality, particularly infection-related mortality from increased toxicity of intensive treatment strategies, is still a significant concern.

Treatment-related mortality (TRM) is poorly defined and reported in studies. Increased accuracy and reporting of TRM and infection-related mortality (IRM) would harmonise results, enabling better comparisons of clinical trials.

Despite the recognised relationship between mucositis and febrile neutropenia, there are no widely used preventative or therapeutic interventions for febrile mucositis. Exploration of possible strategies to reduce IRM and TRM is required.

6.7 What has this thesis contributed?

The TRM study undertaken confirmed the reproducibility and criterion validity of the TRM classification system. This supports the hypothesis that the classification system can be implemented efficiently and effectively in different health care settings, thereby improving the consistency and accuracy of outcome reporting in clinical trials.

The systematic review identified that there is still insufficient evidence to conclude that probiotics are effective and safe in people with cancer. The meta-analysis demonstrated that probiotics might reduce the incidence of diarrhoea, duration of pyrexia and may reduce the incidence of septicaemia and central line infection. Probiotics may be a rare source of infection, but no deaths have been attributed to their consumption.

The MaCROS study demonstrated that it is feasible to undertake a double-blind randomised control clinical trial in this population of patients. The main barriers identified in undertaking an RCT in the future were recruitment and adherence to data capture.

6.8 Future research

6.8.1 Classification of death

Findings from this thesis can be used to guide future research on the reporting of treatment-related mortality and cause of death attribution system. Improved classification and attribution to cause of death would enable improved understanding of

where future research strategies should be targeted to increase overall survival in children with cancer or supportive care strategies.

The TRM study identified the need to refine the approach to categorising cause of death in cancer patients receiving care without intent to cure. This is particularly important if the system is applied in 'routine' settings, such as assessing deaths in a palliative setting, rather than in the original setting of use - a curative trial. Increasingly, individuals destined not to be cured are living for lengthier periods due to participation in clinical trials/studies. This group of patients currently have all deaths classified as "not treatment-related mortality" as clinicians would have specified either progressive disease or that cancer therapy has no curative intent. The algorithm in figure 1 may fail to identify a significant group of patients, who may die of causes amenable to better supportive care, whilst receiving palliative care. This could be addressed by modifying the algorithm for this type of use.

Reviewers in the TRM did not agree on 6 out of 10 causes of death attributed to TRM. Further research should focus on the application of the cause of death attribution system, and on how agreement in outcome reporting could be improved. Understanding and interpretation of the system, as proposed for attribution of a specific mechanistic cause of death, could potentially be improved by dedicating more time during the presentations and using the newly developed web-based training tool.

The author of this thesis has published findings from this study (147). Since this publication the classification tool and cause of death attribution system has been used to analyse data from two Dutch hospitals (21) and a population-based cohort (148).

The use of the TRM classification system and cause of death attribution has been validated in centres in high-income countries. Future research would ideally focus on its use in middle-income countries. This study can help guide future global strategies.

6.8.2 Probiotics

The author of this thesis has published this systematic review and meta-analysis (149). The updated systematic review identified twelve ongoing trials investigating the use of probiotics in people with cancer. An updated systematic review and meta-analysis should, therefore, be undertaken in 3-5 years' time.

As adverse events were also not clearly or uniformly defined in identified studies, it cannot be determined if all relevant data were appropriately identified, recorded or

documented. However, the variability of definitions used and reporting of adverse events means conclusions cannot be drawn with confidence. Further harmonisation of reporting of clinical trials using strategies such as the COMET initiative and CONSORT checklist would enable greater precision and confidence in conclusions drawn.

6.8.3 Paediatric feasibility studies

The MaCROS study demonstrated the feasibility of undertaking an RCT, but that further research is needed to address barriers to success, specifically recruitment and the adherence to the capture of data. The evaluation of the MaCROS study identified a lack of evidence-based targeted interventions, such as the QuinteT intervention or SWAT analysis, to improve recruitment in paediatric studies. Further research should focus on implementing evidence-based targeted interventions in paediatric studies.

The research undertaken in this thesis has identified a paucity of research exploring the biological evidence for the use of probiotics in people with cancer. Assessment of the microbiome, the gut flora, and any colonisation by probiotics observed may help in understanding the interactions between probiotic consumption and mucositis, be it beneficial or ineffective. Using a 'biological tool' to explore the severity of mucositis with the use of probiotics could enhance understanding of the aetiology, stratification and treatment of mucositis. Therefore, biomarkers may be a useful aid to investigate how probiotics may impact mucositis, and it would be beneficial to assess the feasibility of undertaking a parallel sub-study, in a future study, to further understand the biological impact of probiotics in children with febrile mucositis.

6.9 Overall conclusion

This thesis has validated a uniform definition of TRM and disease-progression for the classification of death in children with cancer, which has applicability in clinical trials and health care settings in high-income countries. Findings from this study were presented to the UK National Cancer Research Institute Clinical Studies Research Group. This was favourably received by the research group and is currently being considered at a national and European level.

This thesis has also identified and investigated how probiotics can be used to reduce or prevent mucositis and infection in children diagnosed with cancers, potentially through the modification of the microbiome. Findings from the systematic review and meta-analysis have been used to change recommendations at the Royal Victoria Hospital in

Newcastle. Because of this study, children diagnosed with cancer can now take probiotics as part of their neutropenic diet.

The results of the MaCROS study are currently being disseminated nationally and internationally. Evaluation of the MaCROS study and systematic review have been used to identify facilitators and barriers for undertaking a future randomised control trial and has also been used to develop a parallel biological sub-study. It is hoped this sub-study will be included in a future study.

It is hoped that outcomes from this thesis will shape future research and clinical interventions for the use of probiotics in children diagnosed with cancers.

7 Appendix A

1.1 TRM ethical approval

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Dear Hadeel

Ref no: **MREC15-118**

Title: **Validation of a system for classifying treatment-related mortality in children with cancer**

Your research application has been reviewed by the School of Medicine Ethics Committee (SoMREC) and we can confirm that ethics approval is granted based on the following documentation received from you.

Document	Version	Date Submitted
HHassan ethics form TRM final version 2	2.0	17/08/2016
Participation information leaflet TRM study version 3	3.0	17/08/2016
consent form for TRM study version 3	3.0	17/08/2016
MREC15-118 LTHT R&D Opinion	1.0	01/06/2016
MREC15-118 Signature page completed	1.0	01/06/2016
HHassan TRM protocol final	1.0	29/07/2016
HHassan TRM study participant email	1.0	29/07/2016
HHassan TRM study data extraction form	1.0	22/06/2016
dialogue with Richard Barrett LTHT IG governance	1.0	17/06/2016

Please notify the committee if you intend to make any amendments to the original research ethics application or documentation. All changes must receive ethics approval prior to implementation. Please contact the Faculty Research Ethics Administrator for further information (fmhsresethics@leeds.ac.uk)

Ethics approval does not infer you have the right of access to any member of staff or student or documents and the premises of the University of Leeds. Nor does it imply any right of access to the premises of any other organisation, including clinical areas. The committee takes no responsibility for you gaining access to staff, students and/or premises prior to, during or following your research activities.

Please note: You are expected to keep a record of all your approved documentation, as well as documents such as sample consent forms, and other documents relating to the study. This should be kept in your study file, which should be readily available for audit purposes. You will be given a two week notice period if your project is to be audited.

It is our policy to remind everyone that it is your responsibility to comply with Health and Safety, Data Protection and any other legal and/or professional guidelines there may be.

We wish you every success with the project.

Yours sincerely

Dr Naomi Quinton
Co-Chair, SoMREC, University of Leeds

(Approval granted by Co-Chair Dr Naomi Quinton on behalf of committee)

SoMRECApproval letter v2_0

September 2013

1.2 Participant information Leaflet

Leeds institute of Cancer and Pathology

Version 2

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Part I: Participant Information Leaflet

Validation of a system for classifying treatment-related mortality

We would like to invite you to take part in a research study. Before you decide you need to understand why the research is being done and what it would involve for you. Please take time to read the following information carefully. Please ask us if there is anything that is not clear or if you would like more information.

What is the purpose of the study?

Cause of death in children with cancer may be attributed to the cancer and disease-progression, or complications of treatment delivered. Death not directly due to the cancer has been termed "treatment-related mortality (TRM)" and includes infection, bleeding, and organ dysfunction. Appreciating differences between TRM and disease-related death is critical in helping researchers and clinicians understand how to direct care and therapies to improve survival. A collaboration led by Lillian Sung from Toronto in Canada developed and validated a classification system for TRM intended for use by clinical research assistants (CRA), non-medically qualified professionals who work with clinical trial data capture and entry. Causes of death in identified patients' notes were reviewed using the developed classification tool by two independent CRAs and compared with two consultants. This system requires validation in different centres, to assess if it can be applied consistently outside of the institution in which it was developed. This could potentially be used for international clinical trials enabling uniformity of outcomes reported.

What is the aim of the study?

The aim of this study is to validate the TRM classification at Leeds Teaching Hospital Trust (LTHT), by assessing the agreement of clinical consultants and CRAs. Two consultant paediatric haematologist or oncologists and two CRAs will review 30 anonymised case notes of most recent deaths of patients with malignancy, or who received a haemopoetic stem cell transplant, while under the care of LTHT. The CRAs decisions will be a compared to those of the consultants.

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Why have I been asked to participate?

You are either a consultant oncologist/haematologist or clinical research associate who meets the requirement required for participate who has been identified by the research team

What will happen now?

If you agree to participate in the study, we will ask you to attend a chosen location at Leeds Teaching Hospital Trust in the near future which is suitable for all participants. After signing written consent form you will receive training on how to use the classification tool which will be delivered in person by the research team alongside any relevant documentation required. You will then be placed in separate rooms (without any direct contact or communication with the other participants) and given 60 sets of anonymised case notes. You will then review the notes and classify death according to the algorithm. For the patients whose death you classify as TRM you will assign a probable cause of death according to a further schema. After a short break you will meet with the other participants to discuss your experiences of using the classification tool. Your participation should take approximately half a working day.

Do I have to take part?

You do not have to take part in the study. If you agree to participate we will ask you to sign a written consent form confirming your willingness to participate.

Do I have the right to withdraw from the study?

You have the right to withdraw from the study up to 2 weeks after completion of the study by contacting the study lead with your request. After two weeks you will not be able to withdraw your participation.

Are there any disadvantages of taking part in this study?

If you participate in the study, you will be reviewing notes of children with cancer who have died. This may cause you to become upset or distressed. If you feel you need further support the research team will be able to advise who to contact.

Will my taking part in the study be kept confidential?

Your participation and information supplied is entirely confidential and will not be disclosed to anyone outside the research team without your permission. We also ask

that you keep all study information and discussions confidential also. All the information collected will be stored securely according to the Data Protection Act 1998.

Who is organising and funding the research?

The study is led by Dr Hadeel Hassan a paediatric trainee, and Clinical Research Fellow and PhD student at the University of Leeds under the supervision of Dr Bob Phillips consultant paediatric oncologist and senior academic, and Professor Sally Kinsey, Paediatric haematologist. Dr Hadeel Hassan's Clinical Research Fellowship is funded by The Candlelighters Charity.

Who has reviewed the study?

This study has been reviewed by the University of Leeds, School of Medicine Research Ethics

Committee (SoMREC), reference number MREC15-118."

Who do I contact for further information?

For further information about the study please contact Dr Hadeel Hassan at the University of Leeds telephone number 0113 3432596 or Dr Bob Phillips at Leeds Teaching Hospital 0113 39 28779 (secretary).

Do I have to take part?

You do not have to take part in the study. If you agree to participate we will ask you to sign a written consent form confirming your willingness to participate.

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1.3 Consent form

Leeds institute of Cancer and Pathology Version 2

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Consent to take part in the Validation of a system for classifying treatment-related mortality study

	Add your initials next to the statements you agree with
I confirm that I have read and understand the information sheet dated 29/7/16 explaining the above research project and I have had the opportunity to ask questions about the project.	
I agree for the data collected from me to be stored and used in relevant future research in an anonymised form.	
I understand that relevant sections of the data collected during the study, may be looked at by individuals from the University of Leeds or from regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
I agree to take part in the above research project and will inform the lead researcher should my contact details change.	
I understand that I have the right to withdraw from the study up to 2 weeks after completion of the study by contacting the study lead with my request. After 2 weeks I will not be able to withdraw my	

participation.	

PTO

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Name of participant	
Participant's signature	
Date	
Name of lead researcher	
Signature	
Date*	

***To be signed and dated in the presence of the participant.**

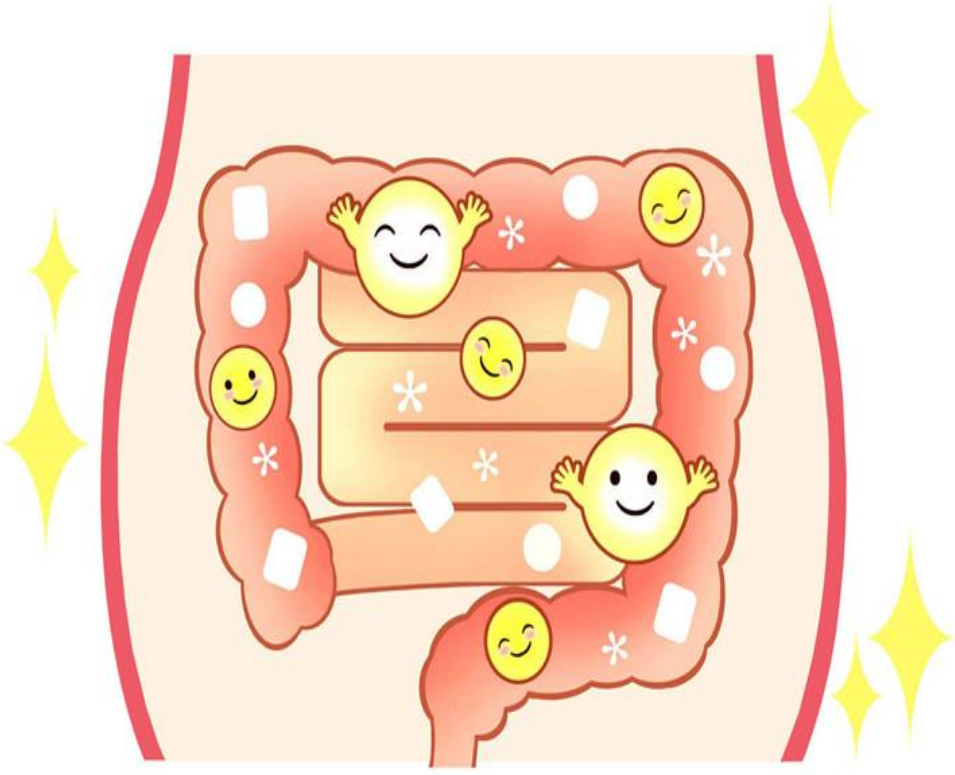
Once this has been signed by all parties the participant should receive a copy of the signed and dated participant consent form, the letter/ pre-written script/ information sheet and any other written information provided to the participants. A copy of the signed and dated consent form should be kept with the project's main documents which must be kept in a secure location.

8 Appendix B

2.1 MaCROS study protocol

Mucositis and infection reduction with liquid probiotics in children with cancer: a randomised-controlled feasibility study protocol

The MaCROS study



Contents

Research reference numbers

IRAS PROJECT ID: 246313

SPONSOR: University of Leeds

CLINICALTRIALS.GOV identifier: NCT03785938

Key trial contacts

Table 21: Key trial contacts

Chief Investigator Principal Investigator	Dr Hadeel Hassan Room 9.86 Level 9, Worsley Building University of Leeds Clarendon Way Leeds LS2 9NL Telephone: 07437319762 Email: umhh@leeds.ac.uk, hadeelhassan@nhs.net
Trial Co-ordinator	Dr Hadeel Hassan Room 9.86 Level 9, Worsley Building University of Leeds Clarendon Way Leeds LS2 9NL Telephone: 07437319762 Email: hadeelhassan@nhs.net umhh@leeds.ac.uk
Sponsor	University of Leeds Clarendon Way Leeds LS2 9NL Telephone: 0113 3434897 Email: governance-ethics@leeds.ac.uk

Funder(s)	Candlelighters charity
Key Protocol Contributors	Dr Bob Phillips Centre for Reviews and Dissemination University of York 01904 321040 Bob.phillips@york.ac.uk Professor Sally Kinsey Leeds Institute of Cancer and Pathology University of Leeds 0113 3928772 Sally.kinsey@nhs.net
Trials Pharmacist	Caroline Bedford Medicines Management and Pharmacy Services Leeds Teaching Hospital Trust 0113 3922459 Caroline.bedford2@nhs.net

Trial summary

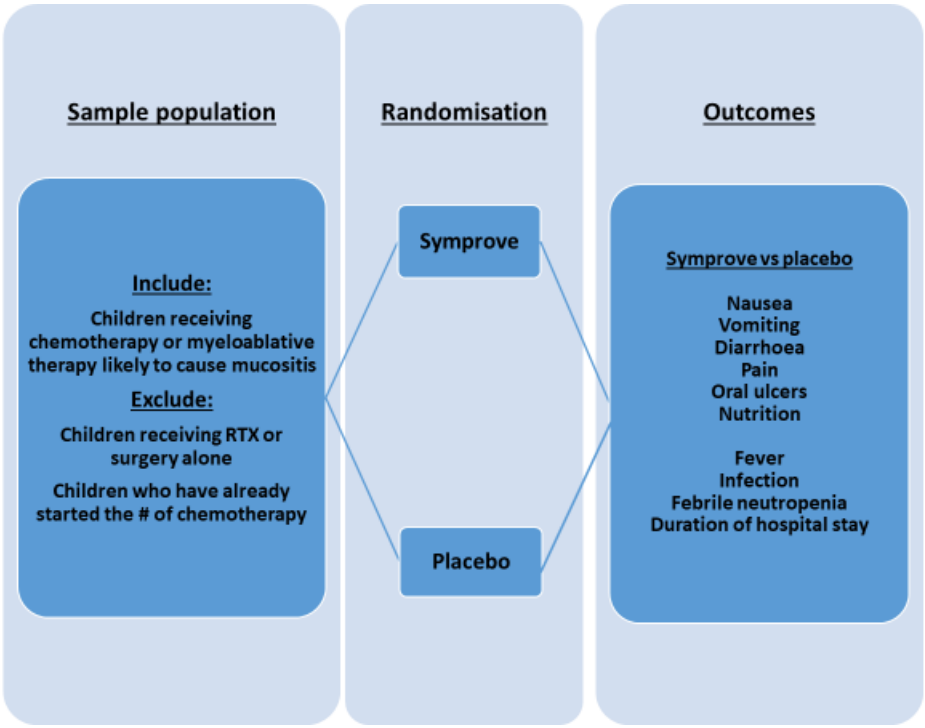
Trial Title	**Mucositis and infection reduction with liquid probiotics: a randomised-controlled feasibility study**	
Internal ref. no. (or short title)	**The MaCROS study**	
Clinical Phase	Feasibility	
Trial Design	Double-blind randomised-controlled trial (RCT)	
Trial Participants	Patients treated on paediatric cancer protocols receiving chemotherapy on regimens likely to cause mucositis	
Planned Sample Size	20-40 participants	
Treatment duration	14 days	
Follow up duration	21 days	
Planned Trial Period	6 months	
	Objectives	Outcome Measures
Primary	Evaluate the feasibility of an RCT to investigate the	Recruitment

	efficacy of liquid probiotics (Symprove) to prevent or reduce mucositis and infection in children diagnosed with cancer undergoing treatment regimens likely to cause mucositis	Completion rates of intervention/placebo Completion rate of symptom diary (paper/web-app) Preliminary health economic evaluation
Secondary	Evaluation of the research protocol Compare outcomes detailed in both groups Explore participants and parents views of experiences	The incidence, severity and duration of diarrhoea The incidence of nausea, vomiting, oral mucositis Recorded telephone or face to face interview of parents Use of analgesia and evaluation of hospital admission
Investigational Product	Symprove liquid probiotic dietary supplement	

Formulation, dose, route of Administration	Symprove liquid probiotic which is available in 2 flavours- Symprove original and mango and passion fruit. Placebo: similar appearance, taste and consistency. The placebo is an identical liquid in appearance and taste, containing distilled water (99.22%), mango and passion fruit natural flavour (0.50%), ascorbic acid (0.26%), and beta-carotene (0.02%). Formulation Taken NG orally, or nasogastric tubing or gastrostomy. Ages: <4 years of age: 20 mls 4-8 years of age: 0.5mls/kg >8 years of age: 1ml/kg

Trial flow chart

Figure 7 : The MaCROS study flow chart



Aim

The aim of this study is to evaluate the feasibility of an RCT to investigate the efficacy of liquid probiotics to prevent or reduce mucositis and infection in children diagnosed with cancer who are undergoing treatment with regimes likely to cause mucositis.

Objectives

The primary objectives of this study will therefore determine:

Whether it is feasible to recruit children diagnosed with cancer who are at risk of developing mucositis

The completion rates of participants taking the liquid probiotic/placebo for 2 weeks

The completion rate of the symptom diary (paper/web-app) by participants/parental to record the symptoms of nausea, vomiting, diarrhoea, oral mucositis and abdominal pain from the start of chemotherapy for 21 days

Preliminary health economic information surrounding the costs/benefits of the intervention.

Secondary objectives will include:

Evaluation of the research protocol

Barriers to compliance with the protocol

Evaluation of intended outcomes to be assessed if an RCT is undertaken. This will include, but is not limited to the incidence, severity and duration of diarrhoea and infection in both groups, the incidence of nausea, vomiting, oral mucositis, and use of analgesia and evaluation of hospital admissions.

Background

Probiotics are defined as “live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host” according to the World Health Organisation and United Nations Food and Agriculture organization (FAO) (37). The most common strains belong to the genera *Lactococcus* and *Bifidobacterium* (38). Health benefits attributed to probiotics include the reduction of the severity of antibiotic associated diarrhoea in paediatric patients(42), necrotising enterocolitis in premature infants (150) and the incidence of radiation-induced diarrhoea (151).

Chemotherapy and radiotherapy induced diarrhoea is a common adverse event and is associated in particular with fluorouracil, capecitabine and irinotecan-based treatment regimes. Radiotherapy is believed to potentially alter bacterial flora and affect the intestinal motility and vascular permeability of mucosal cells (44). Chemotherapy is thought to alter the composition of intestinal flora and therefore affect the metabolism of intestinal enzymes which is vital for gut integrity. Changes to the gut flora may impact the gut defence barrier, immune function and absorption of vital nutrients (45). It is estimated that 20-45% of all chemotherapy patients experience severe diarrhoea (30). Radiotherapy or chemotherapy induced diarrhoea may interrupt or even stop treatment, impair the quality of life and prolong hospital stay of patients with cancer, also potentially increasing health economic burdens (46).

There have been multiple studies investigating the role of probiotics in reducing chemotherapy and radiotherapy associated diarrhoea. An updated systematic review and meta-analysis was undertaken (PROSPERO registration: CRD42016050252). Randomised controlled trials (RCT), identified through screening multiple databases were included for analyses of efficacy. Non-randomised controlled-trials and case reports were included for safety analysis. Outcomes included the reduction in the incidence and severity of diarrhoea, and adverse events. Where possible, data were combined for meta-analysis using a random-effects model.

Twenty one studies (N = 2,982 participants) were included for assessment of efficacy. Results showed probiotics may reduce the incidence of diarrhoea in patients with cancer [odds ratio (OR) = 0.52, 95% confidence interval (CI) 0.34-0.78, 95% percentage prediction interval (PI) 0.3-0.92, I-sq 36.9%, 5 studies], duration of pyrexia [standardized mean difference 0.64 days, 95% CI 053-0.77, PI 0.64-0.64, I-sq 0.01%, 5 studies] and possibly the severity of diarrhoea [for example Common Toxicity Criteria grade 3 and 4 diarrhoea [OR=0.51, 95% CI 0.12-2.2, PI 0.03-9.08, I-sq 92.5%, 4 studies]. Twenty five studies (N = 2,242) were included in the safety analysis. Five case reports showed probiotic-related bacteraemia/fungaemia/ positive blood cultures. The definitions and reporting of adverse events were variable and inconsistent. It was not possible to undertake planned sub-group analyses, investigating age, strains and dosage of probiotic and patient characteristics through marked heterogeneity of study characteristics.

The review demonstrated there were insufficient studies to assess the true effect of probiotics in people with cancer. Meta-analysis suggests probiotics may be beneficial but further studies are still required, particularly in children.

We therefore propose a feasibility study to investigate whether it is possible to undertake a RCT investigating the use of probiotics compared with placebo for preventing and reducing mucositis and infection in children diagnosed with cancer. Data from this study will be used to inform and assess the feasibility of a pragmatic randomised controlled trial. Results will be reported according to the CONSORT 2010 statement for feasibility and pilot studies (152).

Methods

Trial design

This will be a single-centre double-blind randomised-controlled feasibility study.

Study setting

This study at Leeds Teaching Hospital Trust, Leeds UK between January to July 2019.

Recruitment

Participants will be recruited from the paediatric haematology and oncology department at Leeds Teaching Hospital Trust, Leeds, UK.

Target recruitment

The recruitment target is between 20 and 40 patients over a 6 month period. As this is a feasibility study, a power calculation is not required.

Eligibility criteria for participants

Patients between the ages of 1 and 18 treated on paediatric cancer protocols receiving chemotherapy on regimens likely to cause mucositis. These include the UK ALL 11 (patients receiving delayed intensification therapy in regimen A and all participants receiving induction, post induction therapy and delayed intensification in regimen's B and C) IntReALL SR 2010, Inter-B-NHL ritux 2010, Euro Ewings 2012, RMS 2005, SIOP Ependymoma II, Headstart III, SIOPEN HR Neuroblastoma study and Myechild01 protocols. Participants receiving myeloablative therapy will also be included; for example those receiving high dose chemotherapy with stem-cell rescue for high risk neuroblastoma.

Exclusion criteria

Patients who have already started the course of chemotherapy.

Patients receiving radiotherapy or surgery alone.

Patients diagnosed with an immunodeficiency (excluding IgA).

Patients who have previously taken probiotics within the month prior to commencing the course of chemotherapy.

Interventions

Participants will be required to commence the blinded liquid probiotic (Symprove) or placebo on the first day of their chemotherapy/pre stem cell transplant chemotherapy conditioning and take this once daily for 14 days. The dose will be adjusted according to age.

Symprove liquid probiotic

Symprove (Symprove Ltd, Farnham, Surrey, UK) is a liquid probiotic that contains four strains of bacteria with a total of 109 colony forming units: (Lactobacillus rhamnosus NCIMB 30174, Lactobacillus plantarum NCIMB 30173, Lactobacillus acidophilus NCIMB 30175, and Enterococcus faecium NCIMB 30176) in a water-based suspension of barley extract. Symprove is classified as a food supplement under EU law (153).

It is hypothesised that the barley extract suspension, provides acid protection and a nutrient source for the bacteria when compared to freeze-dried probiotic formulations, and a greater number of bacteria survives the transit of gastrointestinal system. This results in a higher number of probiotic bacteria to colonise in the colon (154). Colonisation of bacteria from a probiotic supplement is believed to increase the secretions of anti-inflammatory cytokine interleukins (IL) such as IL-10 and IL-8, T-regulatory cells, and reduce interferons which inhibits the development of oral, intestinal inflammation and therefore mucositis (155).

The use of Symprove in randomised-controlled trials has been investigated in conditions affecting the gastrointestinal system, including inflammatory bowel disease, irritable bowel disease and diverticular disease.

Symprove in adults

Data suggests that Symprove reduces intestinal inflammation in patients with ulcerative colitis (156), and frequency of diarrhoea and mucorrhea in patients with diverticular disease (13). Another randomised controlled trial demonstrated that the use of Symprove resulted in lower symptom severity in participants with irritable bowel syndrome (IBS) when compared to placebo (mean difference −35.0 ,95% CI; −62.03, −7.87; P = 0.01) (157).

Symprove in paediatrics

The use of Symprove has been approved for use in children under EU law and Symprove encourages the use of the liquid probiotics in paediatrics. However, dose

varies in the younger children (20mls for those under the age of 4 and 0.5mg/kg for children aged between 4-8). There have been no paediatric clinical investigating the use of Symprove and the MaCROS study will be the first trial exploring the use of Symprove in paediatrics and people with cancer. It is believed the liquid and flavoured formulations will improve compliance in children when compared to tabled/freeze dried formulations. As the use of Symprove has been investigated in other gastrointestinal disorders the study authors felt it would be an appropriate intervention to explore in children with cancer who are at risk of developing mucositis.

Safety of Symprove

Liquid probiotic has been reported as safe in previous studies undertaken and there have been no reports of serious adverse events attributed to Symprove (153, 158). However, consumption may be associated with nausea and reflux (159). As previously reported probiotics can rarely be associated with infections in immunocompromised patients.

Randomisation: type

Simple randomisation will be undertaken by the research pharmacist due to the small number of participants intended to be recruited. Ideally randomisation would be stratified according to type of malignancy (solid tumour/ leukaemia/ stem cell transplants - allogeneic or autologous), chemotherapy type (etoposide, doxorubicin or high dose methotrexate, SCT), age (dichotomised at 10 years and older).

Randomisation: implementation

Allocation concealment

Health care professionals and participants will be blinded to the randomisation allocation.

Blinding

Patients, health care professionals (apart from the pharmacy department) and the research team will be blinded to the type of intervention delivered (intervention or placebo). The placebo and probiotic will be packaged in identical sealed boxes, identified by a trial batch/code as supplied by the Symprove company.

Data collection

Patient diary

The diary will include questions to assess nausea, vomiting, diarrhoea, pain and oral mucositis (using a modified version of the Children’s International Mucositis Evaluation

Scale (ChIMES) (103)). An example of the patient diary is supplied in the appendix. Either the participant or parent will fill in the diary on a daily basis for a minimum of 21 days. If possible, the same person will be required to fill in the diary during the 21 days. Participants/parents/guardians will have the option of choosing to use a paper diary or a web-app diary. If participants/parents/guardians choose to use the web-app they will be given a link to download the web-app which they filly daily. They can also opt for daily reminders using their mobile number or email address.

Investigation of febrile episodes/infection

Clinical records including electronic and written records will be reviewed to investigate any febrile episodes and infections for incidence and duration of fever/infection and duration of hospital stay until afebrile for 48 hrs.

Other data

Other relevant information including type of nutritional support, analgesia and duration of hospital stay, will be taken from clinical records during any hospital stay. Data collected will be anonymised and stored on data collection forms.

Evaluation of participant/parent experience

Participants and/or parents will be invited to discuss their experiences of participation in the trials. Those agreeing to participate will be interviewed over the phone, or in person depending on preference, and the interviews audio recorded. This will include questions regarding recruitment, the process of gaining consent and randomisation, and experiences of adherence of the probiotic/placebo and patient diary and should not take longer then 45 minutes.

Information will be collected using a recording audio device, transcribed and information will be evaluated using the framework approach [21] . The recording will be deleted as immediately after completion of transcribing. Interviewees will be asked not to refer to any names or patient identifiable information. Information will be used in conjunction with the development of any future RCT.

Participants who chose to decline participation the MaCROS study will also be invited to fill an optional anonymous questionnaire explaining why they chose not to take part in the study. Consent will be implied by return of the questionnaire.

Data management

Information will be collected centrally in the NHS and stored anonymously at the University of Leeds.

Participant/parent to fill in diary daily.

CI to collect diaries following completion of study and collect data of participants admitted to hospital using their clinical records.

Data to be anonymised locally, with only the randomisation number issued by pharmacy will be available on forms.

Any spreadsheet will be anonymised on a secure NHS computer before transfer and access and on a secure password protected University of Leeds computer using the secure NHS email account.

Data supplied on the web-app will only be linked to the participants randomisation number and stored on the secure cloud based Amazon web server. Web-app information will only be accessed on a secure NHS or university of Leeds computer and transferred to anonymised excel sheet which is only identifiable by the participants randomisation number which will be stored on a password protected University of Leeds M Drive. Once data has been stored on the M drive this will be deleted from the web-app server.

Participants/parents/guardians who opt for daily reminders to fill in the app will have information stored on the secure cloud based Amazon web server reminder system. Once the diary has been completed their mobile number/email address will be deleted.

Participants/parents/guardians who agree to participate in interviews will have this recorded using an NHS or university of Leeds encrypted audio recording. Once transcribing has finished the recording will be deleted.

Personal data relating to study to be destroyed by PI or supervisor at end of storage period (10 years).

Consent forms/diaries will be secured in locked filing cabinet in Martin Wing D floor at LTHT and destroyed after the 10 year period.

Electronic database at the University of Leeds will be used to collect the pseudo anonymised data.

Database to be stored on CI University M drive, a secure, password protected, University of Leeds server.

CI or responsible person (e.g. Professor Kinsey) will be responsible for deleting data from database at end of storage period.

Data analysis

Statistical analysis

Statistical power is not required for this feasibility study. However, we believe recruiting between 20-40 participants will supply enough information to evaluate the feasibility of undertaking an RCT.

Data will be entered into a secured local anonymised database and analysed using descriptive statistics, Student's t-test, Mann–Whitney U-test and $\chi 2$ tests for comparative normal, non-normal and categorical data respectively. Where possible appropriate subgroup analysis will be undertaken.

Evaluation of the feasibility for undertaking a randomised-controlled trial

The feasibility of undertaking an RCT will be evaluated using both quantitative and qualitative methods. Data relating to timing of the return of patient diaries, department referral rate, recruitment rate and numbers lost to follow-up will be recorded. Acceptability and tolerability of the treatment intervention will be assessed through completion rate of the probiotic/placebo course, use of the patient diaries and exploration of the patients/parents study participation via interview.

Indications to consider stopping the study

Unable to recruit a minimum of 20 patients within 6 months of the study opening

The occurrence of an unexpected serious adverse reaction or event attributed to the probiotic or placebo

Intentional non-compliance/deviation from the study protocol (e.g. patient un-blinded to intervention delivered without approval from a member of the research team)

Indications to consider modifying the study

Poor recruitment- e.g. fewer than 10 patients recruited within a 3 month period.

Poor identification of eligible patients (less than 100%)

Problems with delivery/compliance 50-80% of intervention or placebo delivered

Poor compliance with recording and returning patient diaries (less than 80%).

Indications to continue the study without modification

No issues implementing study protocol

Adequate number of participants identified and recruited within the 6 month period

100% compliance with the delivery of the intervention/placebo

Greater than 80% compliance of recording and returning of patient diaries.

Indications to not undertake a RCT

Unable to recruit minimum of 20 patients within 6 months of the study opening

Poor compliance with recording and returning patient diaries (less than 50%)

Serious concerns identified during qualitative analysis of participants/parents interview.

Safety reporting

The research and clinical team are responsible for identifying any adverse event. Any serious adverse event (SAE) will be reported to REC using the safety reporting form recommended by the Health Research Authority (http://www.hra.nhs.uk/resources/during-and-after-your-study/progress-and-safety-reporting).

As this feasibility study has been classified as a non-CTIMP study, only reports of Serious Adverse reaction (SAE) that are:

- Related to the study (ie resulted from administration of any of the Symprove/placebo)
- Unexpected (i.e .not listed in the protocol as an expected occurrence)

And Serious Adverse Reactions (SAR):

- An adverse event that is both serious and, in the opinion of the reporting investigator, believed with reasonable probability to be due to one of the trial treatments, based on the information provided.

Information should be submitted to the REC using the Non-CTIMP safety report (appendix 1). These should be sent within 15 days of the chief investigator becoming aware of the event. Reports of unexpected SAE/SARs should be unblinded.

2) The University of Leeds sponsors should be notified of any unexpected SAE or SARs within 24 hrs.

Expected SARs

Fever

Febrile neutropenia

Neutropenia

Infections (not attributed to the probiotic)

Haematological toxicity (e.g. low haemoglobin or platelets)

Gut toxicity, mucositis, stomatitis (e.g. mouth ulcers, nausea, vomiting and diarrhoea)

Allergic/anaphylactic reactions (not attributed to the probiotic/placebo)

Vaso-occulsive disease

Pain

Expected SAEs

Hospitalisation, including prolonged stay (which is not attributed to the probiotic/placebo)

Admission to the paediatric intensive care unit (which is not attributed to the probiotic/placebo)

Disease-progression

Expected death due to disease

Dissemination

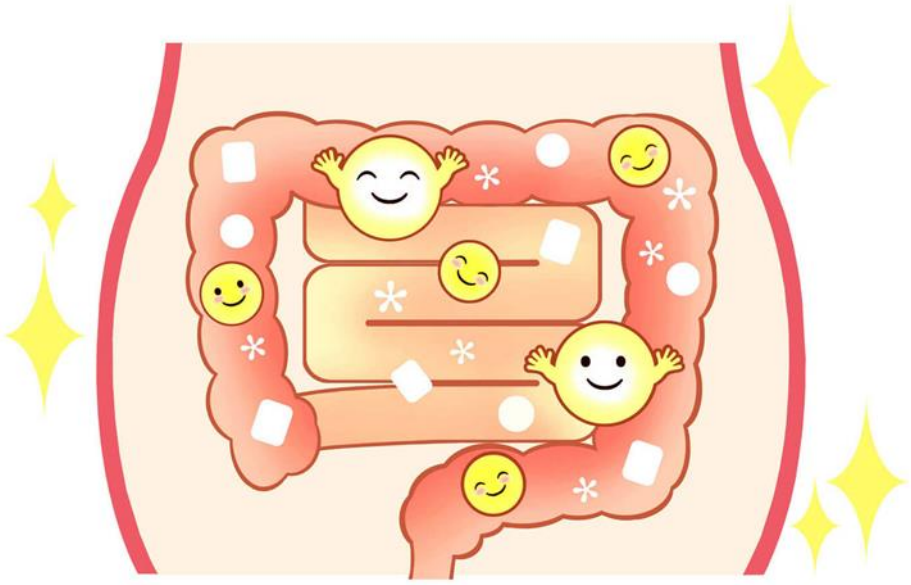
The results of this feasibility study will inform the planning of a definitive RCT, by assessing rates of recruitment, retention, serious adverse events and data collection. The qualitative results will be used to further refine the protocol for a large RCT and presented to potential collaborators. Findings will be presented at relevant meetings as well as manuscripts submitted to peer-reviewed journals according to the CONSORT extension (152).

2.2 PILS for under 10 years

Version 1 03/01/2019 **Study Title: The MaCROS study**

Reference: IRAS no 246313

The MaCROS study



Mucositis and infection reduction with probiotics in children with cancer: a randomised-controlled feasibility study

Participant Information Leaflet for children

Chemo Duck

Takes Part In The MaCROS Study

Hi, I’m Chemo Duck

I finished the MaCROS study last week.

Shall I tell you what it was like for me?

When I came to hospital to have the medicine that makes me better, one of the nice doctors asked if I would take an extra medicine for 2 weeks.

This extra medicine has friendly bugs called ‘bacteria’ which may help your tummy or mouth feel better. This is called a ‘probiotic’.

It's pretty cool because some of other chemo ducks took a different extra medicine. They took a 'pretend' probiotic medicine!

I was told some chemo ducks had the 'pretend' probiotic medicine because the doctors don't know if the probiotic really works so they need to do some detective work to find out!

Guess what..! I didn't know if I was taking the 'pretend' or the probiotic as is a secret..!

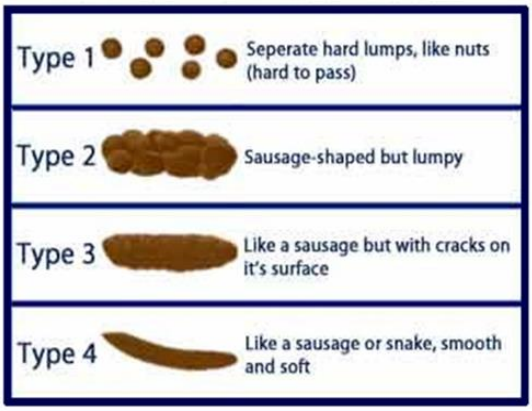
Taking the medicine

The extra medicine I took was a drink. It either had a fruity or plain taste. It looked like juice!

I had to take it every day for 2 weeks.

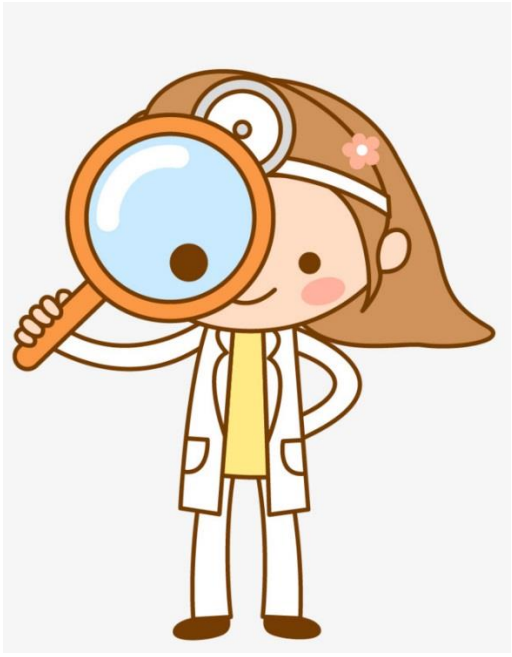
Every day for 3 weeks I had to answer questions about any tummy or mouth problems or if it was hurting anywhere in a diary.

The doctor said that whoever looked after me at home (like mummy or daddy) could answer the questions if I didn't want to, but I wanted to do it because there was some funny pictures like this:



The detective work

The doctor collected my diary once I had finished filling it in.



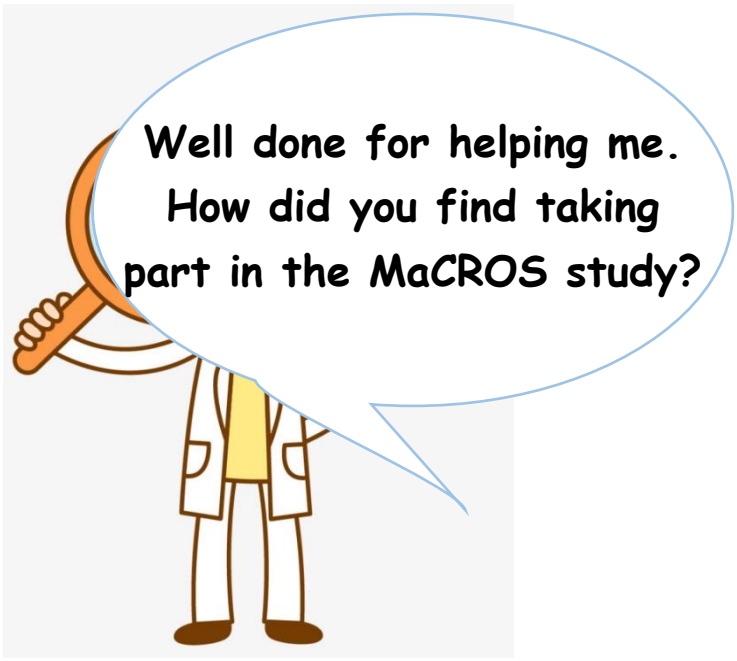
The doctor told me they will investigate my diary and hospital notes from when I had to go to the ward because I had a temperature.

She said she will look at all the other chemo's duck's diaries and hospital notes.

The doctor will collect the diaries and compare the secret groups to see if the probiotic can help make other chemo ducks better.

The doctor told me that chemo ducks who took the probiotic may poo less.!

The hospital and university may keep some of the information about you in the MaCROS study for up to 10 years after it finishes. If you want to find out more about this you can ask your parents or any of the doctors or nurses.

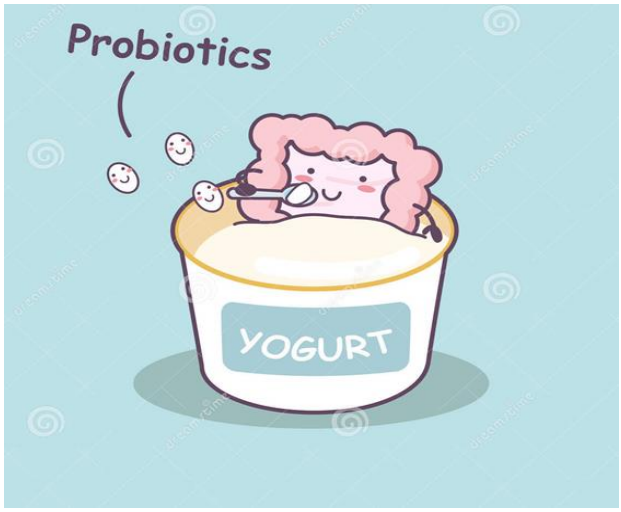



I hope it makes other chemo ducks better!

2.3 PILS 10-16 years

Mucositis and infection reduction with probiotics in children with cancer:

a randomised-controlled feasibility study



The MaCROS study

Participant Information Leaflet for persons aged 10 to 16

Will you receive chemo which may affect your gut?

Would you be interested in taking part in a study which *may* reduce your symptoms AND help other kids or teenagers in the future?

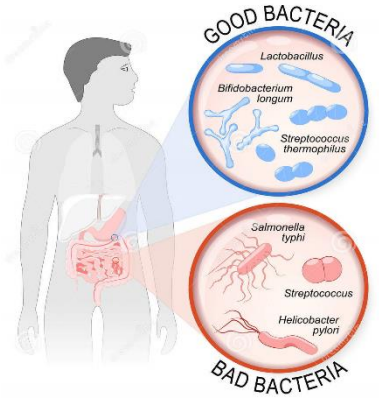
If so The MaCROS study maybe the study for you…

What are probiotics?

Probiotics are supplements which contain 'friendly bacteria' which can help your gut.

It is believed that taking probiotics may reduce some of the side effects of the chemo you are having.

This includes:



Mouth ulcers

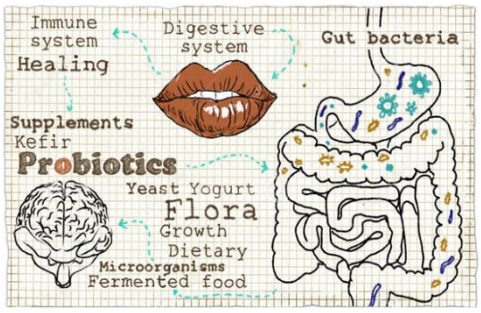
Tummy pain

Sickness

Vomiting

Poo problems

Infections



However, we don't have enough *proof to* show this. Research (studies) is a way of trying to find answers to questions we don't know the answer to.

What will the MaCROS study investigate?

The MaCROS study will explore if it is worth doing a large study called a 'randomised-controlled trial' (RCT). This study will investigate how practical the study is and whether children and teenagers *actually* want to get involved. The study will also compare side effects described above in children and teenagers who take probiotics and those who take a 'dummy probiotic' called a placebo.

Why have I been asked to take part?

You have been asked to take part because you have cancer and receiving chemotherapy which can cause gut problems. You can help us find answers that will enable us to better understand if other children would be able to take part in the study in the future

Did anyone else check the study is okay to do?

This study has been checked by several people to make sure it is alright

Do I have to take part?

No you don't. It is your choice whether you want to take part in the study and you can always change your mind. We will only collect information until the point you change your mind.

What will I have to do if I get involved?

A doctor will have a chat with you about the MaCROS study and answer any questions you may have. Once you have had time to think and if you decide to take part, yourself and a parent or guardian will be asked to sign a consent form.

You will be asked to start taking the probiotic or the placebo on the first day of your course of chemo. No one apart from someone in pharmacy will know which type you are taking.

You will need to take this every day for two weeks.

You will also be given a symptom diary and will need to fill it in every day from the start of your course of chemo for three weeks. This can filled in using a paper diary or a web-app diary which can be downloaded using a link provided. If you decide to use the app you can also chose to have daily reminders. The diary should take no longer then 15 minutes per day to fill in.

This will involve answering questions about:

Pain

Ulcers

Nausea

Vomiting

Poo problems

One of the doctors (Dr Hadeel Hassan) will look at your hospital records if you end up developing a temperature and get admitted to hospital for antibiotics.

Dr Hadeel will collect your diary when you have finished the diary.

You may be invited to give your opinions on taking part of the study. This can be done on the phone or in person (whatever you prefer!) It shouldn't take any longer than 45 minutes. It will be recorded using a secure device. What is discussed will be transferred (anonymously) to a word document which will be kept on a password protected secured device. Once this has been done the audio recording will be deleted.

What are the potential benefits of taking probiotics?

You may have no benefit from taking the probiotic or placebo.

However, there has been a lot of interest in probiotics in the medical world.

It is already used to prevent infection in vulnerable premature babies.

It has also be shown to reduce diarrhoea in children and teenagers taking antibiotics

It *may* improve:

Pain

Ulcers

Sickness

Diarrhoea

Are there any risks with taking probiotics?

Probiotics have been reported to be very safe.

25 studies were reviewed to investigate how safe it is for people with cancer to take probiotics.

Side effects reported were bloating and sickness.

In very very *rare* cases a person may get an infection which is caused by the friendly bacteria. The few who did get an infection made a full recovery with antibiotic treatment.

What will happen when the research study stops?

The research will be talked about and written down but no one will know that you took part. All identifiable information about you will be kept private. Non-identifiable

information will be kept at the university of Leeds for up to 10 years on a secure computer. This non -identifiable information maybe used in future studies.

The pharmacy team will also keep some identifiable information (i.e. name, NHS number and what you received). This will only be kept on a secure NHS computer for up to 10 years.

What if something goes wrong?

If there is a problem you can you can talk to your parents or guardians, or any of the researchers or health care team. If you don't want to take part anymore then you can tell your parents/guardians or any of the researchers. You don't need to give a reason.

Who is paying for this study?

The Candlelighters charity is paying Dr Hadeel's university degree (called a PhD) and study costs.

More information

For further information you can email ask for Dr Hadeel via any staff member

The NHS website also has some information about probiotics:

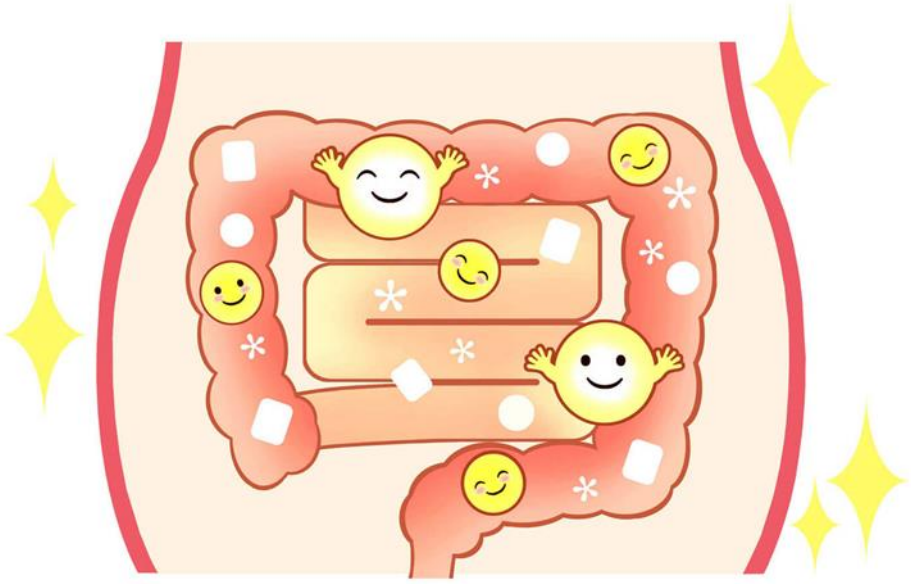
https://www.nhs.uk/conditions/probiotics/

If you wish to make a complaint you can inform Dr Hadeel, any member of the health care team or contact PALS.

Thank you for reading this. Please ask any questions if you need to.

2.4 PILS 16-18 yrs

Mucositis and infection reduction with probiotics in children with cancer: a randomised-controlled feasibility study



The MaCROS study

Participant Information Leaflet for ages 16-18

We would like to invite you to take part in a research study. Before you decide, you need to understand why this research is being done and what it would involve for you. Please take time to read the following information carefully and do not hesitate to ask any questions.

Why probiotics?

There has been a lot of interest regarding the use of probiotics (tablets or granules containing 'friendly bacteria') to improve the health of people with certain medical conditions. It is believed that probiotics can help change the type of bacteria that live in the gut to more 'friendly' types which are good for health. Clinical trials have shown that the use of probiotics may reduce antibiotic-associated diarrhoea in children, gut problems in people with chronic bowel conditions and prevent life threatening gut infections in premature babies.

Certain studies have suggested that probiotics may also reduce side effects of chemotherapy that can affect the gut. This includes diarrhoea, pain, nausea, vomiting,

mouth ulcers and infection. However, there have only been two small clinical trials in children and young people with cancer, and we are therefore not clear if probiotics will work for this group of patients.

What is this study about?

This study will investigate whether a large clinical trial (known as a randomised-controlled trial) should take place. Patients taking part will be randomly allocated to receive the probiotic or a 'dummy probiotic' (known as a placebo). This has to be taken daily from the start of chemotherapy for 14 days. Patients will be asked to answer questions in a diary about problems which may affect the gut on a daily basis for 3 weeks. We will also review records from any hospital admissions during this time to investigate any infections. This is called a *feasibility study.*

Why is a feasibility study needed?

It is important to do a feasibility study before undertaking a randomised-controlled trial as these types of studies are expensive, need a large number of patients, and involve significant time and effort to run. Researchers and health-care professionals may be interested in a particular question, but actually patients or parents may think that the question being asked is not important or actually useful. Sometimes it can be difficult to get patients to agree to take part in a study, take medications or fill in a diary. It is therefore important to identify any issues before the larger study. We will 'trial' the study on fewer patients than that required for the randomised-controlled trial. Information from this feasibility study will be used to guide whether we should proceed with the randomised-controlled trial, and to make the study more patient and family friendly. Essentially this feasibility study is a 'mini randomised-controlled trial' to check everything works and runs smoothly.

Why have I been asked to participate?

You have been diagnosed with cancer, and is receiving chemotherapy which may cause mouth ulcers, nausea, vomiting, pain and infections.

What will happen now?

If you agree to participate in the study, we will ask you to sign a written consent form. Before deciding you will also have the opportunity to ask the research team questions.

Once you have agreed to take part, you will be randomised to receive the probiotic or placebo. This will be designed and packaged so that you or health care professionals will not be able to tell whether it is the probiotic or placebo. However, it will be labelled in a way that the research team will be able to identify what has been given. The

reason why we are doing this is to try reduce any issues which could cause you, researchers and health care professionals to misinterpret results (in research this is known as bias).

You should take the probiotic or placebo every day from the start of chemotherapy for 14 days. If you find that you cannot take the probiotic or placebo, then please do inform a health care professional or a member of the research team.

We also ask that you answer the questions in the diary (paper or web-app version) every day for 3 weeks once you start taking the probiotic or placebo. If you chose to use the web-app you will be given a web-app link to down load. You can log into the app using the identification number issued by pharmacy on your bottle of probiotic or placebo. You also have the optional choice of supplying your mobile number/email address to receive daily reminders to fill in the diary but you do not have to do this.

You will also have the option to participate in an interview over the phone or in person. This will involve answering questions about your experiences. This should not take any longer than 45 minutes. The interview will be recorded using a secure audio device which will then be transferred to an anonymized word document. Once this has been completed the audio recording will be deleted.

Anonymous quotes from interviews may be used during the analysis and reporting of the study (for example PhD thesis or journal papers).

Taking part in the interview will help us understand how to improve the patient experience in any future study but you do not have to do this to take part in the MaCROS study. It is an optional part of the study.

Why is it important to do this?

You may develop side effects 10-14 days after taking chemotherapy. We have attempted to time the probiotic or placebo to fit in with this to see whether it could prevent or reduce any side effects. A randomised-controlled trial would investigate whether this is the correct time to give the probiotic, as well as the necessary duration of probiotic for any beneficial effect, and how easy or difficult it is to fill in the diary for 21 days. The feasibility study will help us decide if this is a realistic thing to ask patients or parents to do.

What are the benefits of taking part in this study?

You may receive no benefit from taking part in this study.

You will be contributing to a study which may improve the quality of life of children with cancer. Whilst the main goal of this study is to investigate if we should undertake a

randomised-controlled trial, you may benefit from fewer side effects including diarrhoea, pain, nausea, vomiting, mouth ulcers and infection.

Do I have to take part?

You do not have to take part in the study. If you agree to participate we will ask you and/or your parent to sign a written consent form confirming your willingness to participate.

Can I change my mind after I have agreed to take part?

You can withdraw at any point during the study.

Are there any disadvantages of taking part in this study?

As with any supplement or medicine there is a very small risk of developing side effects. Studies suggest that there are very few side-effects that can occur when a patient takes probiotics whilst receiving chemotherapy. There is a small risk of developing an infection which is caused by the 'friendly bacteria' in the probiotic. However, this is a very rare side effect. In a large review of 25 studies investigating the use of probiotics in people with cancer only 6 out of 1138 patients (0.5%) developed any probiotic-associated infection. All patients made a full recovery and the probiotic associated infections were successfully treated with antibiotics and supportive care. Other symptoms such as vomiting, bloating and diarrhoea were reported in both the probiotic and placebo groups. These symptoms are usually associated with the chemotherapy being used.

While on the study you will receive the same clinical care as if you were not on the study. If you develop a temperature or became unwell you would be reviewed, investigated and treated as required for standard care. This would include attending hospital, being reviewed by health care professionals, having the usual investigations and receiving IV antibiotics or any other treatments required. In the unlikely development of a probiotic associated infection, appropriate antibiotics will be delivered following discussion with the microbiologists (doctors who give advice on antibiotics choice when targeting a particular bacteria). A safety and risk analysis will also be undertaken by the research team.

Is this study insured?

This MaCROS study is insured and has indemnity covered by the University of Leeds.

Will my taking part in the study be kept confidential?

We will inform your GP that you are participating in the MaCROS study. Otherwise, participation and any information supplied is entirely confidential and will not be disclosed to anyone outside the research team without your permission.

Your participation and any information supplied is entirely confidential and will not be disclosed to anyone outside the research team without your permission. We also ask that you keep all study information and discussions confidential too. All the information collected will be stored securely according to the Data Protection Act 2018.

The university of Leeds will keep non-identifiable information about you for 10 years. This data will be stored on a secure password protected computer and will only be accessible by a member of the research team responsible about data. Information may be used in future studies but only non-personalised and non-identifiable data will be supplied.

The lead research pharmacist will also have your name and unique identifiable number on a database which is password protected on a secure NHS computer. This will be deleted after 10 years.

Consent forms and paper diaries will be kept in a locked cabinet in the paediatric haematology and oncology office in Leeds General Infirmary.

Will I be contacted about the results of the MaCROS study?

If we receive your consent we can send you a letter reporting the outcome of the study once it has closed.

Transparency

The University of Leeds is the sponsor for this study based in the United Kingdom. We will be using information from your medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your information and using it properly. The university of Leeds will keep identifiable information about you for the purpose of the study for up to 10 years after the study has finished. This information will be held by the Leeds Teaching Hospital Trust.

Your rights to access, change or move your information are limited, as we need to manage your information in specific ways in order for the research to be reliable and

accurate. If you withdraw from the study, we will keep the information about you that we have already obtained. To safeguard your rights, we will use the minimum personally-identifiable information possible.

Leeds Teaching Hospital Trust will collect information from your medical records for this research study in accordance with our instructions.

Leeds Teaching Hospital Trust (LTHT) will keep your name, NHS number and contact details. LTHT will not pass this information to the University of Leeds. LTHT will use this information as needed, to contact you about the research study, and make sure that relevant information about the study is recorded for your care, and to oversee the quality of the study. Certain individuals from the University of Leeds and regulatory organisations may look at your medical and research records to check the accuracy of the research study. The University of Leeds will only receive information without any identifying information. The people who analyse the information will not be able to identify you and will not be able to find out your name, NHS number and contact details.

LTHT will keep identifiable information about you from this study for up to 10 years after the study has completed.

You can find out more about how we use your information by contacting the University of Leeds data protection officer on DPO@leeds.ac.uk

Who is organising and funding the research?

The study is led by Dr Hadeel Hassan, a paediatric trainee and Clinical Research Fellow and PhD student at the University of Leeds under the supervision of Dr Bob Phillips, Consultant paediatric oncologist and senior academic, and Professor Sally Kinsey, Paediatric haematologist. Dr Hadeel Hassan's Clinical Research Fellowship is funded by The Candlelighters Charity.

Who has reviewed the study?

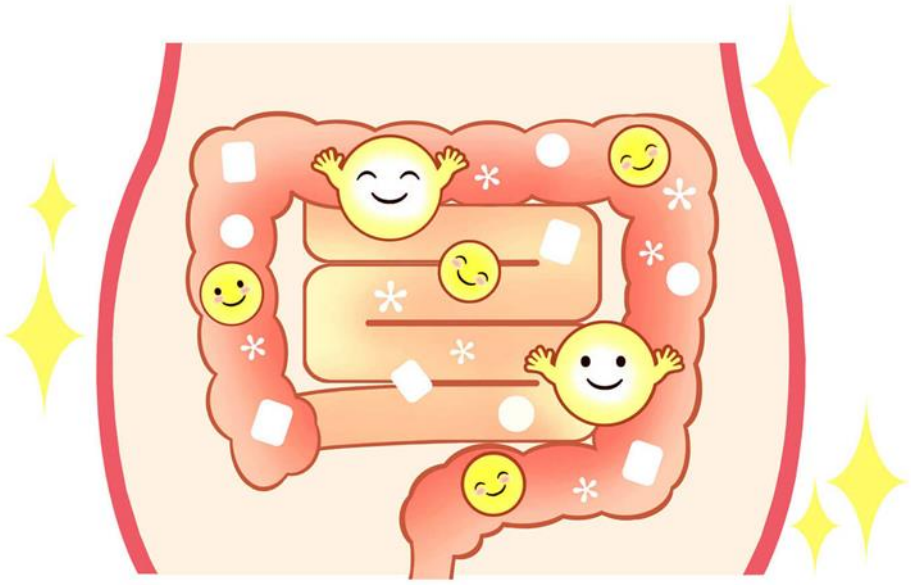
The NHS Research Ethics service has reviewed this study to ensure to ensure your rights, safety, dignity and well-being are protected whilst facilitating research.

Who do I contact for further information? For further information about the study please contact Dr Hadeel Hassan at the University of Leeds, telephone number 0113 3432596, or Dr Bob Phillips at Leeds Teaching Hospital 0113 39 28779 (secretary).

If you wish to log a complaint about this study you can do so by contacting PALS.

2.5 PILS parents/guardians

Mucositis and infection reduction with probiotics in children with cancer: a randomised-controlled feasibility study



The MaCROS study

Participant Information Leaflet for person with parental/responsibility

We would like to invite your child to take part in a research study. Before you decide, you need to understand why this research is being done and what it would involve for your child. Please take time to read the following information carefully and do not hesitate to ask any questions.

Why probiotics?

There has been a lot of interest regarding the use of probiotics (tablets or granules containing 'friendly bacteria') to improve the health of people with certain medical conditions. It is believed that probiotics can help change the type of bacteria that live in the gut to more 'friendly' types which are good for health. Clinical trials have shown that the use of probiotics may reduce antibiotic-associated diarrhea in children, gut

problems in people with chronic bowel conditions and prevent life threatening gut infections in premature babies.

Certain studies have suggested that probiotics may also reduce side effects of chemotherapy that can affect the gut. This includes diarrhoea, pain, nausea, vomiting, mouth ulcers and infection. However, there have only been two small clinical trials in children and young people with cancer, and we are therefore not clear if probiotics will work for this group of patients.

What is this study about?

This study will investigate whether a large clinical trial (known as a randomised-controlled trial) should take place. Patients taking part will be randomly allocated to receive the probiotic or a 'dummy probiotic' (known as a placebo). This has to be taken daily from the start of chemotherapy for 14 days. Patients or those with parental responsibility will be asked to answer questions in a diary about problems which may affect the gut on a daily basis for 3 weeks. We will also review records from any hospital admissions during this time to investigate any infections. This is called a *feasibility study.*

Why is a feasibility study needed?

It is important to do a feasibility study before undertaking a randomised-controlled trial as these types of studies are expensive, need a large number of patients, and involve significant time and effort to run. Researchers and health-care professionals may be interested in a particular question, but actually patients or parents may think that the question being asked is not important or actually useful. Sometimes it can be difficult to get patients to agree to take part in a study, take medications or fill in a diary. It is therefore important to identify any issues before the larger study. We will 'trial' the study on fewer patients than that required for the randomised-controlled trial. Information from this feasibility study will be used to guide whether we should proceed with the randomised-controlled trial, and to make the study more patient and family friendly. Essentially this feasibility study is a 'mini randomised-controlled trial' to check everything works and runs smoothly.

Why have I been asked to participate?

Your child has been diagnosed with cancer, and is receiving chemotherapy which may cause mouth ulcers, nausea, vomiting, pain and infections.

What will happen now?

If you agree for your child to participate in the study, we will ask you to sign a written consent form. Before deciding you will also have the opportunity to ask the research team questions. If your child is under the age of 16 they may also be asked to sign an assent form agreeing to their participation.

Once you have agreed for your child to take part, your child will be randomised to receive the probiotic or placebo. This will be designed and packaged so that you or health care professionals will not be able to tell whether it is the probiotic or placebo. However, it will be labelled in a way that the pharmacist will be able to identify what has been given if there is any urgent need to know what is given . The reason why we are doing this is to try reduce any issues which could cause you, researchers and health care professionals to misinterpret results (in research this is known as bias).

Your child should take the probiotic or placebo every day from the start of chemotherapy for 14 days. If you find that your child cannot take the probiotic or placebo, then please do inform a health care professional or a member of the research team.

We also ask that you answer the questions about your child in the diary (paper or web-app version) every day for 3 weeks once you start taking the probiotic or placebo. This should not take more than 10 minutes each day. If you chose to use the web-app you will be given a web-app link to download. You can log into the app using the identification number issued by pharmacy on your child's bottle of probiotic or placebo. You also have the optional of receive daily push notifications to fill in the diary but you do not have to do this.

You will also have the option to participate in an interview over the phone or in person. This will involve answering questions about yours and your child's experiences. This should not take any longer than 45 minutes. The interview will be recorded using a secure audio device which will then be transferred to an anonymized word document. Once this has been completed the audio recording will be deleted.

Anonymous quotes from interviews may be used during the analysis and reporting of the study (for example PhD thesis or journal papers).

Taking part in the interview will help us understand how to improve the patient experience in any future study but your child does not have to do this to take part in the MaCROS study. It is an optional part of the study.

Why is it important to do this?

Your child may develop side effects 10-14 days after taking chemotherapy. We have attempted to time the probiotic or placebo to fit in with this to see whether it could prevent or reduce any side effects. A randomised-controlled trial would investigate whether this is the correct time to give the probiotic, as well as the necessary duration of probiotic for any beneficial effect, and how easy or difficult it is to fill in the diary for 21 days. The feasibility study will help us decide if this is a realistic thing to ask patients or parents to do.

What are the benefits for my child by taking part in this study?

Your child may receive no benefit from taking part in this study.

Your child will be contributing to a study which may improve the quality of life of children with cancer. Whilst the main goal of this study is to investigate if we should undertake a randomised-controlled trial, your child may benefit from fewer side effects including diarrhoea, pain, nausea, vomiting, mouth ulcers and infection.

Does my child have to take part?

Your child does not have to take part in the MaCROS study. If you decide you would like to participate then we will ask you to sign a written consent form on behalf of your child. Your child may also be asked to sign an assent form.

Can I change my mind after I have agreed to take part?

You can withdraw your child at any point during the study.

Are there any disadvantages for my child if we agree to take part in this study?

As with any supplement or medicine there is a very small risk of developing side effects. Studies suggest that there are very few side-effects that can occur when a patient takes probiotics whilst receiving chemotherapy. There is a small risk of developing an infection which is caused by the 'friendly bacteria' in the probiotic. However, this is a very rare side effect. In a large review of 25 studies investigating the use of probiotics in people with cancer only 6 out of 1138 patients (0.5%) developed any probiotic-associated infection. All patients made a full recovery and the probiotic associated infections were successfully treated with antibiotics and supportive care. Other symptoms such as vomiting, bloating and diarrhoea were reported in both the probiotic and placebo groups. These symptoms are usually associated with the chemotherapy being used.

While on the study your child will receive the same clinical care as if they are not on the study. If your child develops a temperature or became unwell they would be reviewed, investigated and treated as required for standard care. This would include attending hospital, being reviewed by health care professionals, having the usual investigations and receiving IV antibiotics or any other treatments required. In the unlikely development of a probiotic associated infection, appropriate antibiotics will be delivered following discussion with the microbiologists (doctors who give advice on antibiotics choice when targeting a particular bacteria). A safety and risk analysis will also be undertaken by the research team.

Will I be contacted about the results of the MaCROS study?

If we receive your consent we can send you a letter reporting the outcome of the study once it has closed.

Will my child's participation in the study be kept confidential?

We will inform your GP that your child is participating in the MaCROS study. Otherwise, participation and any information supplied is entirely confidential and will not be disclosed to anyone outside the research team without your permission.

The university of Leeds will keep non-identifiable information about your child for 10 years. This data will be stored on a secure password protected computer and will only be accessible by a member of the research team responsible about data. Information may be used in future studies but only non-personalised and non-identifiable data will be supplied.

The lead research pharmacist will also have your childs name and unique identifiable number on a database which is password protected on a secure NHS computer. This will be deleted after 10 years.

Consent forms and paper diaries will be kept in a locked cabinet in the paediatric haematology and oncology office in Leeds General Infirmary.

We also ask that you keep all study information and discussions confidential too. All the information collected will be stored securely according to the Data Protection Act 2018.

Is this study insured?

This MaCROS study is insured and has indemnity covered by the University of Leeds.

Transparency

The University of Leeds is the sponsor for this study based in the United Kingdom. We will be using information from your child's medical records in order to undertake this study and will act as the data controller for this study. This means that we are responsible for looking after your child's information and using it properly. The university of Leeds will keep identifiable information about your child for the purpose of the study for up to 10 years after the study has finished. This information will be held by the Leeds Teaching Hospital Trust.

Your rights to access, change or move your child's information are limited, as we need to manage your information in specific ways in order for the research to be reliable and accurate. If you withdraw your child from the study, we will keep the information about your child that we have already obtained. To safeguard your child's rights, we will use the minimum personally-identifiable information possible.

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LTHT will keep identifiable information about your child from this study for up to 10 years after the study has completed.

You can find out more about how we use your child's information by contacting the University of Leeds data protection officer on DPO@leeds.ac.uk

Who is organising and funding the research?

The study is led by Dr Hadeel Hassan, a paediatric trainee and Clinical Research Fellow and PhD student at the University of Leeds under the supervision of Dr Bob Phillips, Consultant paediatric oncologist and senior academic, and Professor Sally

Kinsey, Paediatric haematologist. Dr Hadeel Hassan's Clinical Research Fellowship is funded by The Candlelighters Charity.

Who has reviewed the study?

The NHS Research Ethics service has reviewed this study to ensure to ensure your rights, safety, dignity and well-being are protected whilst facilitating research.

Who do I contact for further information? For further information about the study please contact Dr Hadeel Hassan at the University of Leeds, telephone number 0113 3432596, or Dr Bob Phillips at Leeds Teaching Hospital 0113 39 28779 (secretary).

If you wish to log a complaint about this study you can do so by contacting PALS.

2.6 Consent forms parents/guardians

Mucositis and infection reduction with probiotics in children with cancer: a randomised-controlled feasibility study protocol

Consent form for those with parental responsibility

Name:

	Add your initials next to the statements you agree with
I confirm that I have read and understand the information sheet version ……. dated …….. explaining the above research study I have had the opportunity to ask questions about the project.	
I understand that relevant anonymised quotes from myself or my child may be used in published works arising from this study	
I agree for the data collected from my child to be stored securely and used in relevant future research in an anonymised form.	
I understand that relevant sections of the data collected during the study, may be looked at by individuals from the University of Leeds or from regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to my child's records.	
I understand that I have the right to withdraw my child from the MaCROS study at any point	
I am happy for my child or myself to be approached about participating in a telephone/face to face	

UNIVERSITY OF LEEDS

interview about my experiences of participating in the MaCROS study.	
I understand that the interview will be recorded on an audio device and deleted as soon as the interview has been transcribed to a word document.	
I am happy for my child's GP to be informed of his/her participation in The MaCROS study.	
I would like to be contacted about the result of the MaCROS study by letter once it has closed	
I give permission for my child to participate in the MaCROS study.	

Name of person with parental responsibility	
Signature	
Date	
Name of health care professional/ research professional	
Signature	
Date*	

Once this has been signed by all parties the participant should receive a copy of the signed and dated participant consent form, the letter/ pre-written script/

information sheet and any other written information provided to the participants. A copy of the signed and dated consent form should be kept with the project's main documents which must be kept in a secure location.

2.7 Consent forms 16-18 yrs

Mucositis and infection reduction with probiotics in children with cancer: a randomised-controlled feasibility study protocol

Consent form for 16-18 yr olds

Name:

	Add your initials next to the statements you agree with
I confirm that I have read and understand the information sheet version ……. dated …….. explaining the above research study I have had the opportunity to ask questions about the project.	
I understand that relevant anonymised quotes may be used in published works arising from this study	
I agree for the data collected from me to be stored securely and used in relevant future research in an anonymised form.	
I understand that relevant sections of the data collected during the study, may be looked at by individuals from the University of Leeds or from regulatory authorities where it is relevant to my taking part in this research. I give permission for these individuals to have access to my records.	
I understand that I have the right to withdraw from at any point	
I am happy to be approached about participating in a telephone/face to face interview about my experiences of participating in the MaCROS study.	
I understand that the interview will be recorded on an audio device and deleted as soon as the	

interview has been transcribed to a word document.	
I am happy for my GP to be informed of my participation in The MaCROS study	
I consent to participating in the MaCROS study	
I would like to be contacted about the result of the MaCROS study by letter once it has closed	

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Signature	
Date	
Name of health care professional/ research professional	
Signature	
Date*	

Once this has been signed by all parties the participant should receive a copy of the signed and dated participant consent form, the letter/ pre-written script/ information sheet and any other written information provided to the participants. A copy of the signed and dated consent form should be kept with the project's main documents which must be kept in a secure location.

2.8 Assent form

NHS
Health Research Authority

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The MaCROS study version 1 03/1/2019

ASSENT FORM for children under the age of 16

Mucositis and infection reduction with liquid probiotics in children with cancer: a randomised-controlled feasibility study protocol

ASSENT FORM for children under the age of 16

By responsible health care/research professional

	Responsible health care/research professional initials next to the statements you agree with
I have checked with the child understands that participation is voluntary	
I have checked with the child and they understand the procedures	
I have checked with the child and they understand the risks and discomforts	
I have checked with the child and they understand the benefits_____ (initial)	

By child

	Add your initials next to the statements you agree

	with
I know that I can choose to be in the research study or choose not to be in the research study. I know that I can stop whenever I want. **I have read this information (or had the information read to me) and I understand it.** **I have had my questions answered and know that I can ask questions later if I have them.** **I understand any changes to this will be discussed with me.** **I agree to take part in the research.**	
I do not wish to take part in the research and I have not signed the assent below	

<u>Certificate of assent</u>

Name of child	
Child/minor's signature	
Date	
Name of health care professional/ research professional	
Signature	
Date*	

Once this has been signed by all parties the participant should receive a copy of the signed and dated participant consent form, the letter/ pre-written script/ information sheet and any other written information provided to the participants. A copy of the signed and dated consent form should be kept with the project's main documents which must be kept in a secure location.

2.9 GP notification letter

The MaCROS Study version 1 03/01/2019

GP LETTER

date

Dear Dr _____,

RE: Patient's Name: _____ DOB: ____/____/___

Address: _____

_____

The MaCROS study

IRAS Number: 246313

This patient has consented to participate in the above clinical trial.

The MaCROS is a randomised controlled feasibility study of probiotics vs placebo. The aim of the study is to evaluate the feasibility of a randomised controlled trial to investigate the efficacy of liquid probiotics to prevent or reduce mucositis and infection in children diagnosed with cancer who are undergoing treatment with regimes likely to cause mucositis.

Patients will be randomised to receive the liquid probiotic or placebo on day 1 -14 of their chemotherapy. Patients, carers and healthcare professionals will be masked as to the allocation. Symptoms will be documented daily in a patient diary.

Outcomes investigated will include analysis of the compliance of participants taking the probiotic on a daily basis and completion of patient diaries (using paper and web-app methods). Departmental referral and recruitment rates, numbers lost to follow up and evaluation of the participant/parent experience using telephone interviews will also be explored. Secondary outcomes investigated will include analysis of the incidence and severity of mucositis and infection, through the use of patient recorded diaries and review of clinical records.

Findings of the completed study will be used to assess if it is feasible to undertake a RCT to investigate whether probiotics can reduce the incidence and severity of mucositis and infection in children with cancer

A copy of the patient-information sheet is inclosed with this letter.

Data protection

Your patient will be followed up for a maximum of 3 weeks or until their discharge from hospital if admitted during the 3 weeks following completion of the probiotic course.

Please keep the research team informed if the patient is hospitalised or in the event of patient death.

Should you have any questions concerning the patient's participation or their treatment, please contact myself, on:

Tel 07437319762 or 01133928488

Dr Hadeel Hassan, Paediatric registrar and Chief investigator

Please ensure that a copy of this letter is kept in the patient's file at your practice and that the patient is flagged as taking part in a clinical trial.

Many thanks and best wishes

Dr Hadeel Hassan

Paediatric registrar, clinical research fellow and

Chief investigator

2.10 Diary: web-app

Access the website URL: www.macrosstudy.com

Access settings tab top right corner

Select add to Home screen

Save as MaCROS study.

2.11 REC approval (REF: 18/YH/0005)

NHS
Health Research Authority

Yorkshire & The Humber - Leeds West Research Ethics Committee
NHSBT Newcastle Blood Donor Centre
Holland Drive
Newcastle upon Tyne
NE2 4NQ

Telephone: 0207 104 8086

Please note: This is the favourable opinion of the REC only and does not allow you to start your study at NHS sites in England until you receive HRA Approval

20 March 2019

Dr Hadeel Hass
Flat 1 St Ann's Hill
81 St Ann's Lane
LS4 2SG

Dear Dr Hass

Study title:	**Mucositis and infection reduction with liquid probiotics in children with cancer: a randomised-controlled feasibility study protocol**
REC reference:	**18/YH/0005**
Protocol number:	**Not applicable**
IRAS project ID:	**248313**

Thank you for your letter of 6th March 2019, responding to the Committee's request for further information on the above research and submitting revised documentation.

The further information has been considered on behalf of the Committee by the Chair.

We plan to publish your research summary wording for the above study on the HRA website, together with your contact details. Publication will be no earlier than three months from the date of this opinion letter. Should you wish to provide a substitute contact point, require further information, or wish to make a request to postpone publication, please contact hra.studyregistration@nhs.net outlining the reasons for your request.

Confirmation of ethical opinion

On behalf of the Committee, I am pleased to confirm a favourable ethical opinion for the above research on the basis described in the application form, protocol and supporting documentation as revised, subject to the conditions specified below.

Conditions of the favourable opinion

The REC favourable opinion is subject to the following conditions being met prior to the start of the study.

Management permission must be obtained from each host organisation prior to the start of the study at the site concerned.

Management permission should be sought from all NHS organisations involved in the study in accordance with NHS research governance arrangements. Each NHS organisation must confirm through the signing of agreements and/or other documents that it has given permission for the research to proceed (except where explicitly specified otherwise).

Guidance on applying for HRA and HCRW Approval (England and Wales)/ NHS permission for research is available in the Integrated Research Application System, at www.hra.nhs.uk or at http://www.rdforum.nhs.uk.

Where a NHS organisation's role in the study is limited to identifying and referring potential participants to research sites ("participant identification centre"), guidance should be sought from the R&D office on the information it requires to give permission for this activity.

For non-NHS sites, site management permission should be obtained in accordance with the procedures of the relevant host organisation.

Sponsors are not required to notify the Committee of management permissions from host organisations

Registration of Clinical Trials

All clinical trials (defined as the first four categories on the IRAS filter page) must be registered on a publically accessible database within 6 weeks of recruitment of the first participant (for medical device studies, within the timeline determined by the current registration and publication trees).

There is no requirement to separately notify the REC but you should do so at the earliest opportunity e.g. when submitting an amendment. We will audit the registration details as part of the annual progress reporting process.

To ensure transparency in research, we strongly recommend that all research is registered but for non-clinical trials this is not currently mandatory.

If a sponsor wishes to request a deferral for study registration within the required timeframe, they should contact hra.studyregistration@nhs.net. The expectation is that all clinical trials will be registered, however, in exceptional circumstances non registration may be permissible with prior agreement from the HRA. Guidance on where to register is provided on the HRA website.

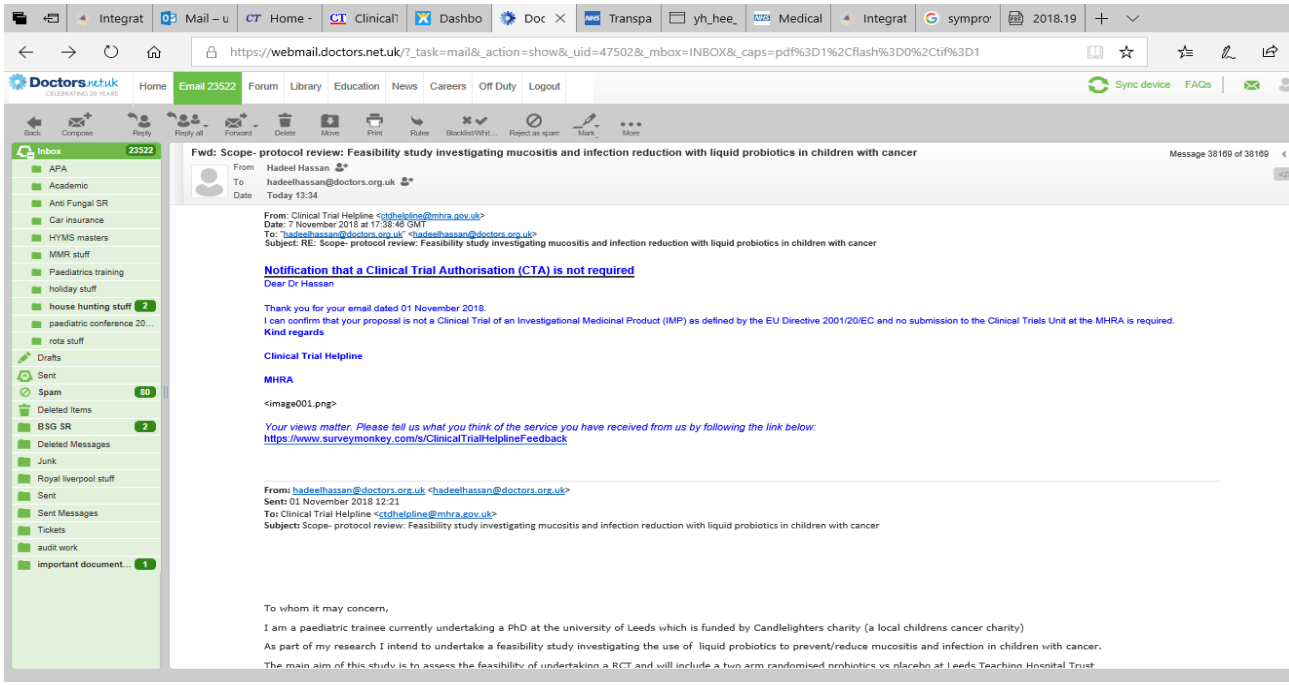
It is the responsibility of the sponsor to ensure that all the conditions are complied with before the start of the study or its initiation at a particular site (as applicable).

Ethical review of research sites

NHS sites

The favourable opinion applies to all NHS sites taking part in the study, subject to management permission being obtained from the NHS/HSC R&D office prior to the start of the study (see "Conditions of the favourable opinion" below).

2.12 MHRA conformation that MaCROS is an non-CTIMP study



Fwd: Scope- protocol review: Feasibility study investigating mucositis and infection reduction with liquid probiotics in children with cancer

From: Hadeel Hassan
To: hadeelhassan@doctors.org.uk
Date: Today 13:34

From: Clinical Trial Helpline <ctdhelpline@mhra.gov.uk>
Date: 7 November 2018 at 17:38:46 GMT
To: "hadeelhassan@doctors.org.uk" <hadeelhassan@doctors.org.uk>
Subject: RE: Scope- protocol review: Feasibility study investigating mucositis and infection reduction with liquid probiotics in children with cancer

Notification that a Clinical Trial Authorisation (CTA) is not required

Dear Dr Hassan

Thank you for your email dated 01 November 2018.
I can confirm that your proposal is not a Clinical Trial of an Investigational Medicinal Product (IMP) as defined by the EU Directive 2001/20/EC and no submission to the Clinical Trials Unit at the MHRA is required.
Kind regards

Clinical Trial Helpline

MHRA

<image001.png>

Your views matter. Please tell us what you think of the service you have received from us by following the link below:
https://www.surveymonkey.com/s/ClinicalTrialHelplineFeedback

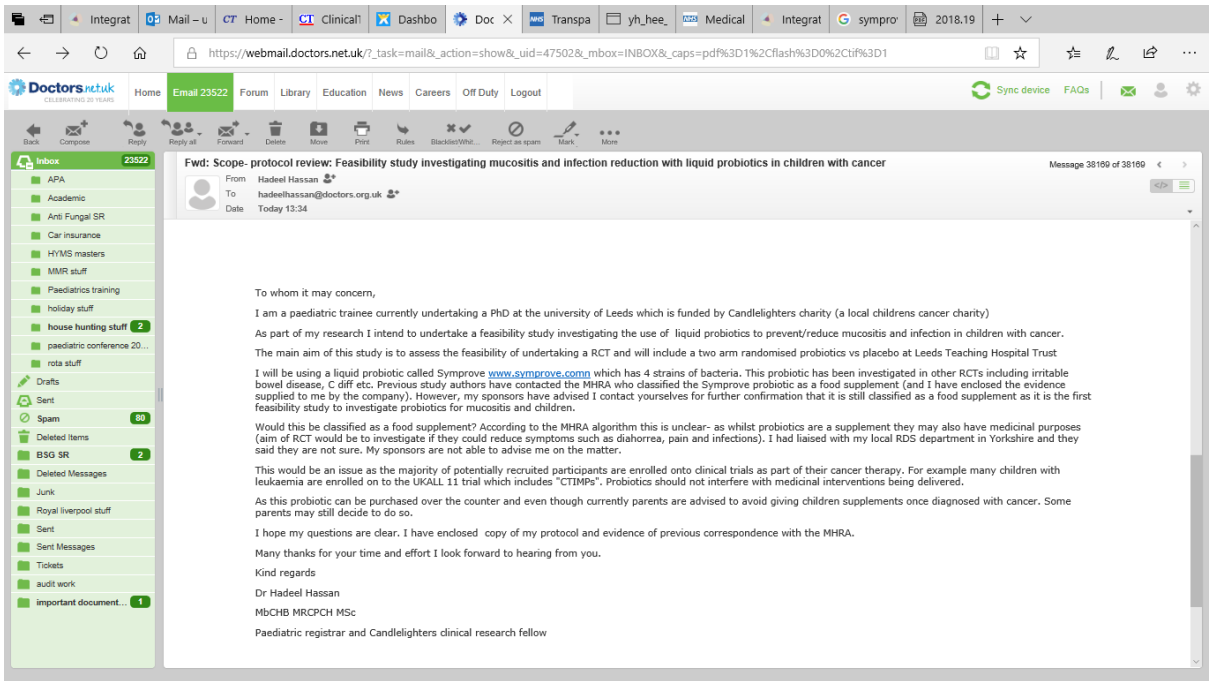
From: hadeelhassan@doctors.org.uk <hadeelhassan@doctors.org.uk>
Sent: 01 November 2018 12:21
To: Clinical Trial Helpline <ctdhelpline@mhra.gov.uk>
Subject: Scope- protocol review: Feasibility study investigating mucositis and infection reduction with liquid probiotics in children with cancer

To whom it may concern,

I am a paediatric trainee currently undertaking a PhD at the university of Leeds which is funded by Candlelighters charity (a local childrens cancer charity)

As part of my research I intend to undertake a feasibility study investigating the use of liquid probiotics to prevent/reduce mucositis and infection in children with cancer.

The main aim of this study is to assess the feasibility of undertaking a RCT and will include a two arm randomised probiotics vs placebo at Leeds Teaching Hospital Trust



Fwd: Scope- protocol review: Feasibility study investigating mucositis and infection reduction with liquid probiotics in children with cancer

From: Hadeel Hassan
To: hadeelhassan@doctors.org.uk
Date: Today 13:34

To whom it may concern,

I am a paediatric trainee currently undertaking a PhD at the university of Leeds which is funded by Candlelighters charity (a local childrens cancer charity)

As part of my research I intend to undertake a feasibility study investigating the use of liquid probiotics to prevent/reduce mucositis and infection in children with cancer.

The main aim of this study is to assess the feasibility of undertaking a RCT and will include a two arm randomised probiotics vs placebo at Leeds Teaching Hospital Trust

I will be using a liquid probiotic called Symprove www.symprove.comn which has 4 strains of bacteria. This probiotic has been investigated in other RCTs including irritable bowel disease, C diff etc. Previous study authors have contacted the MHRA who classified the Symprove probiotic as a food supplement (and I have enclosed the evidence supplied to me by the company). However, my sponsors have advised I contact yourselves for further confirmation that it is still classified as a food supplement as it is the first feasibility study to investigate probiotics for mucositis and children.

Would this be classified as a food supplement? According to the MHRA algorithm this is unclear- as whilst probiotics are a supplement they may also have medicinal purposes (aim of RCT would be to investigate if they could reduce symptoms such as diahorrea, pain and infections). I had liaised with my local RDS department in Yorkshire and they said they are not sure. My sponsors are not able to advise me on the matter.

This would be an issue as the majority of potentially recruited participants are enrolled onto clinical trials as part of their cancer therapy. For example many children with leukaemia are enrolled on to the UKALL 11 trial which includes "CTIMPs". Probiotics should not interfere with medicinal interventions being delivered.

As this probiotic can be purchased over the counter and even though currently parents are advised to avoid giving children supplements once diagnosed with cancer. Some parents may still decide to do so.

I hope my questions are clear. I have enclosed copy of my protocol and evidence of previous correspondence with the MHRA.

Many thanks for your time and effort I look forward to hearing from you.

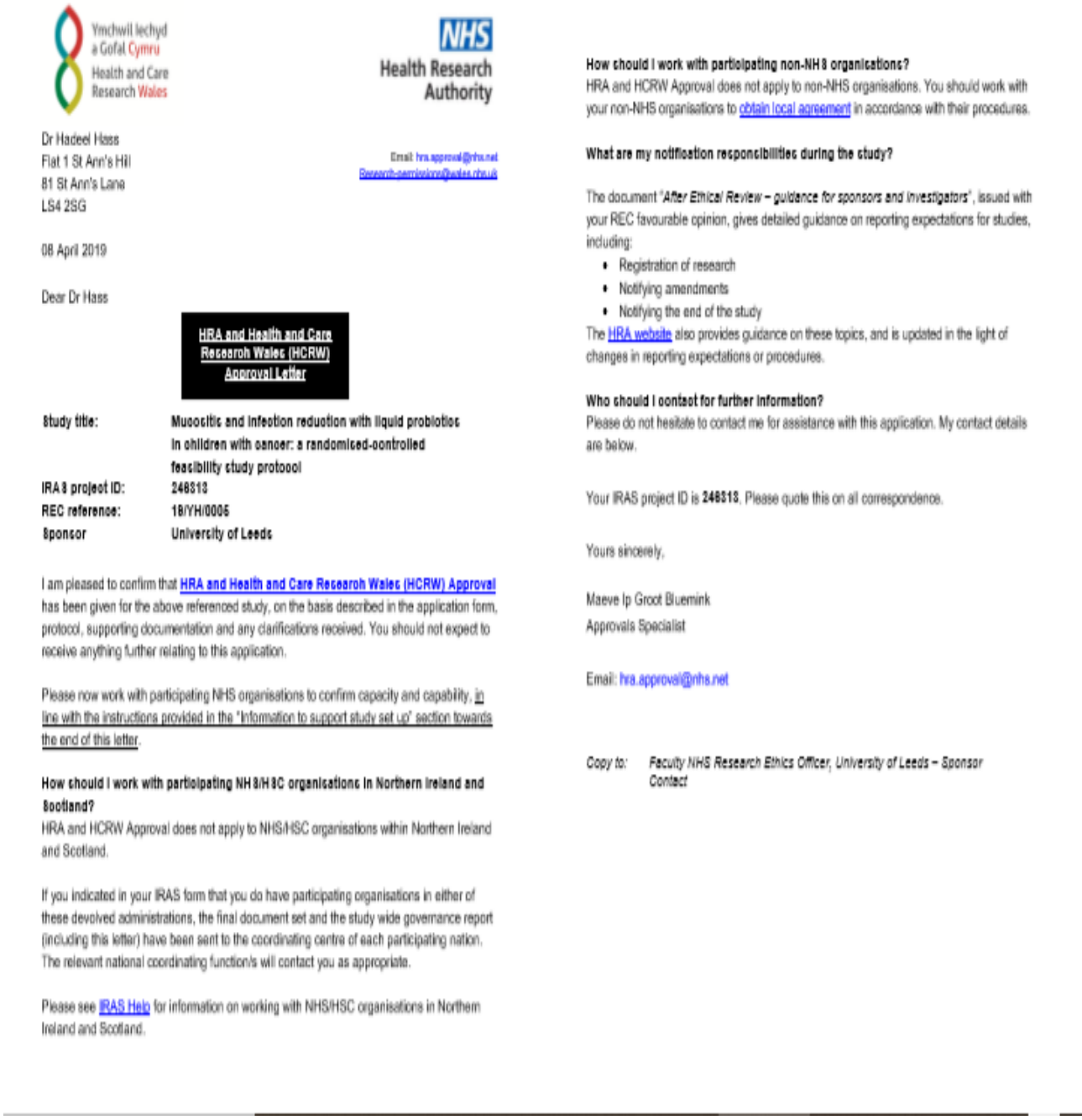
Kind regards

Dr Hadeel Hassan

MbCHB MRCPCH MSc

Paediatric registrar and Candlelighters clinical research fellow

2.13 HRA approval letter



Ymchwil Iechyd a Gofal Cymru
Health and Care Research Wales

NHS
Health Research Authority

Dr Hadeel Hass
Flat 1 St Ann's Hill
81 St Ann's Lane
LS4 2SG

Email: hra.approval@nhs.net
Research.permissions@wales.nhs.uk

08 April 2019

Dear Dr Hass

HRA and Health and Care Research Wales (HCRW) Approval Letter

Study title:	**Mucositis and infection reduction with liquid probiotics in children with cancer: a randomised-controlled feasibility study protocol**
IRAS project ID:	**248313**
REC reference:	**18/YH/0005**
Sponsor	**University of Leeds**

I am pleased to confirm that **HRA and Health and Care Research Wales (HCRW) Approval** has been given for the above referenced study, on the basis described in the application form, protocol, supporting documentation and any clarifications received. You should not expect to receive anything further relating to this application.

Please now work with participating NHS organisations to confirm capacity and capability, in line with the instructions provided in the "Information to support study set up" section towards the end of this letter.

How should I work with participating NHS/HSC organisations in Northern Ireland and Scotland?
HRA and HCRW Approval does not apply to NHS/HSC organisations within Northern Ireland and Scotland.

If you indicated in your IRAS form that you do have participating organisations in either of these devolved administrations, the final document set and the study wide governance report (including this letter) have been sent to the coordinating centre of each participating nation. The relevant national coordinating function/s will contact you as appropriate.

Please see IRAS Help for information on working with NHS/HSC organisations in Northern Ireland and Scotland.

How should I work with participating non-NHS organisations?
HRA and HCRW Approval does not apply to non-NHS organisations. You should work with your non-NHS organisations to obtain local agreement in accordance with their procedures.

What are my notification responsibilities during the study?

The document "*After Ethical Review – guidance for sponsors and investigators*", issued with your REC favourable opinion, gives detailed guidance on reporting expectations for studies, including:

- Registration of research
- Notifying amendments
- Notifying the end of the study

The HRA website also provides guidance on these topics, and is updated in the light of changes in reporting expectations or procedures.

Who should I contact for further information?
Please do not hesitate to contact me for assistance with this application. My contact details are below.

Your IRAS project ID is **248313**. Please quote this on all correspondence.

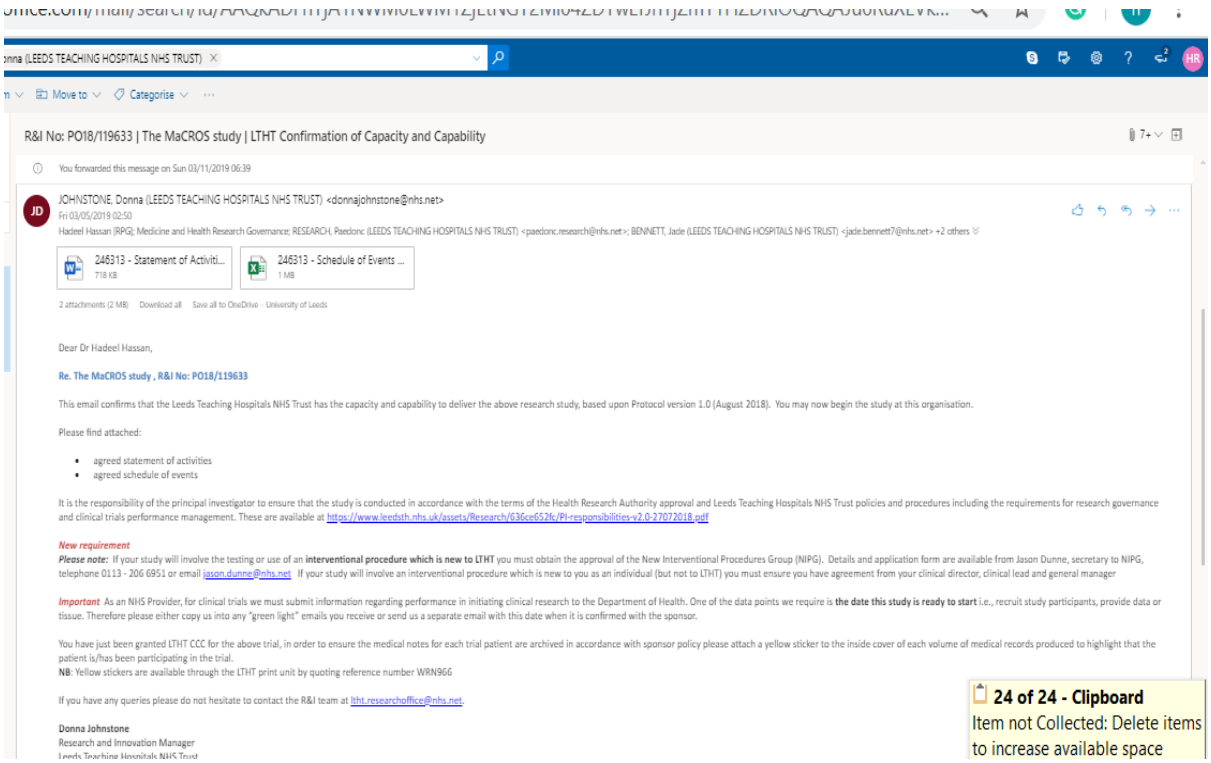
Yours sincerely,

Maeve Ip Groot Bluemink
Approvals Specialist

Email: hra.approval@nhs.net

Copy to: *Faculty NHS Research Ethics Officer, University of Leeds – Sponsor Contact*

2.14 Evidence of LTHT confirmation of capacity and capability



R&I No: PO18/119633 | The MaCROS study | LTHT Confirmation of Capacity and Capability

You forwarded this message on Sun 03/11/2019 06:39

JOHNSTONE, Donna (LEEDS TEACHING HOSPITALS NHS TRUST) <donnajohnstone@nhs.net>
Fri 03/05/2019 02:50
Hadeel Hassan (RPG); Medicine and Health Research Governance; RESEARCH, Paedionc (LEEDS TEACHING HOSPITALS NHS TRUST) <paedionc.research@nhs.net>; BENNETT, Jade (LEEDS TEACHING HOSPITALS NHS TRUST) <jade.bennett7@nhs.net> +2 others

246313 - Statement of Activiti... 718 KB
246313 - Schedule of Events ... 1 MB

2 attachments (2 MB) Download all Save all to OneDrive - University of Leeds

Dear Dr Hadeel Hassan,

Re. The MaCROS study , R&I No: PO18/119633

This email confirms that the Leeds Teaching Hospitals NHS Trust has the capacity and capability to deliver the above research study, based upon Protocol version 1.0 (August 2018). You may now begin the study at this organisation.

Please find attached:

- agreed statement of activities
- agreed schedule of events

It is the responsibility of the principal investigator to ensure that the study is conducted in accordance with the terms of the Health Research Authority approval and Leeds Teaching Hospitals NHS Trust policies and procedures including the requirements for research governance and clinical trials performance management. These are available at https://www.leedsth.nhs.uk/assets/Research/636ce652fc/PI-responsibilities-v2.0-27072018.pdf

New requirement
Please note: If your study will involve the testing or use of an **interventional procedure which is new to LTHT** you must obtain the approval of the New Interventional Procedures Group (NIPG). Details and application form are available from Jason Dunne, secretary to NIPG, telephone 0113 - 206 6951 or email jason.dunne@nhs.net If your study will involve an interventional procedure which is new to you as an individual (but not to LTHT) you must ensure you have agreement from your clinical director, clinical lead and general manager

Important As an NHS Provider, for clinical trials we must submit information regarding performance in initiating clinical research to the Department of Health. One of the data points we require is **the date this study is ready to start** i.e., recruit study participants, provide data or tissue. Therefore please either copy us into any "green light" emails you receive or send us a separate email with this date when it is confirmed with the sponsor.

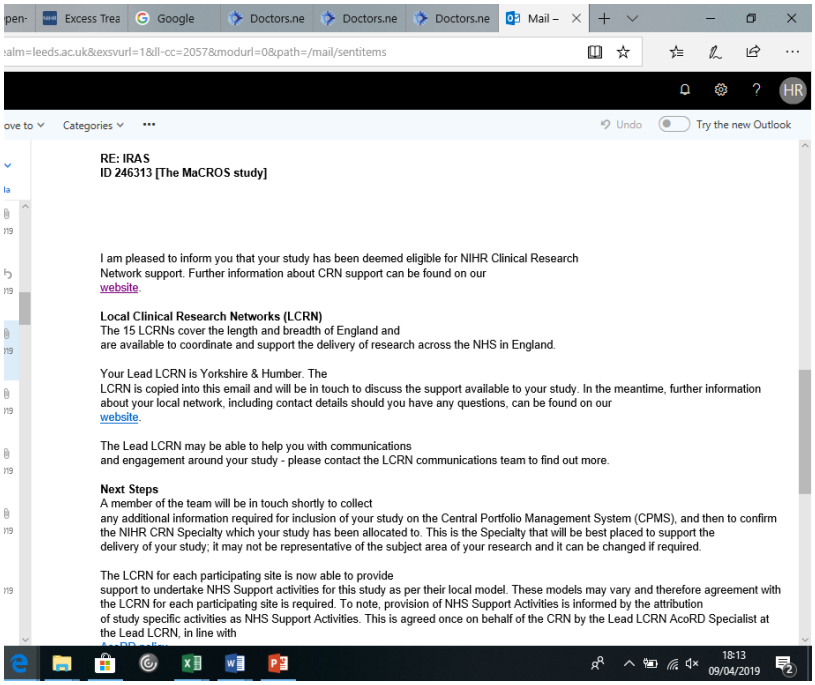
You have just been granted LTHT CCC for the above trial, in order to ensure the medical notes for each trial patient are archived in accordance with sponsor policy please attach a yellow sticker to the inside cover of each volume of medical records produced to highlight that the patient is/has been participating in the trial.
NB: Yellow stickers are available through the LTHT print unit by quoting reference number WRN966

If you have any queries please do not hesitate to contact the R&I team at ltht.researchoffice@nhs.net.

Donna Johnstone
Research and Innovation Manager
Leeds Teaching Hospitals NHS Trust

24 of 24 - Clipboard
Item not Collected: Delete items to increase available space

2.15 Evidence of NIHR portfolio status



RE: IRAS
ID 246313 [The MaCROS study]

I am pleased to inform you that your study has been deemed eligible for NIHR Clinical Research Network support. Further information about CRN support can be found on our website.

Local Clinical Research Networks (LCRN)
The 15 LCRNs cover the length and breadth of England and are available to coordinate and support the delivery of research across the NHS in England.

Your Lead LCRN is Yorkshire & Humber. The LCRN is copied into this email and will be in touch to discuss the support available to your study. In the meantime, further information about your local network, including contact details should you have any questions, can be found on our website.

The Lead LCRN may be able to help you with communications and engagement around your study - please contact the LCRN communications team to find out more.

Next Steps
A member of the team will be in touch shortly to collect any additional information required for inclusion of your study on the Central Portfolio Management System (CPMS), and then to confirm the NIHR CRN Specialty which your study has been allocated to. This is the Specialty that will be best placed to support the delivery of your study; it may not be representative of the subject area of your research and it can be changed if required.

The LCRN for each participating site is now able to provide support to undertake NHS Support activities for this study as per their local model. These models may vary and therefore agreement with the LCRN for each participating site is required. To note, provision of NHS Support Activities is informed by the attribution of study specific activities as NHS Support Activities. This is agreed once on behalf of the CRN by the Lead LCRN AcoRD Specialist at the Lead LCRN, in line with

2.16 Paper diary (Nb sample of cover and day 1. Days 2-21 are repeats of day 1)

What is your User ID?

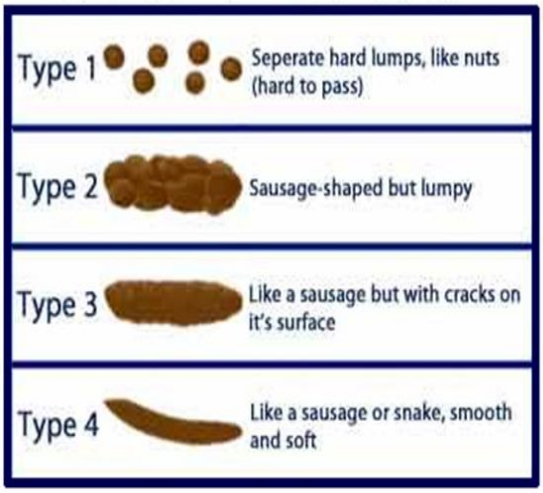
Who filled in the diary? (please tick one):

☐ **Participant**

☐ **Person with parental responsibility**

Day 1

Which of these 7 pictures mostly describes your poo today? Tick the image that matches.



How many times have you pooed today?

Tick on 10, if more than 10

0	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**

Which of these faces describe how bad the nausea is today? Range from 1 (no nausea) to 4 (worst nausea). Tick the option that matches.

How many times did you vomit today?

Tick on 10, if more than 10

0	1	2	3	4	5	6	7	8	9	10

Which of these faces describes how much pain your child feels today? Circle one.

Which of these faces describes how hard it is for your child to eat because of pain? Circle one.

Which of these faces describes how hard it is for your child to drink because of pain? Circle one.

Please look in your child's mouth. Can you see any mouth sores (ulcers)?

☐ Yes ☐ No

Did you feel pain? If so where did you feel it?

Type your answer here:

What medication have you taken today?

☐ Pain, e.g. dihydrocodiene or oramorph

☐ Sickness e.g. ondansetron or metoclopramide

☐ Diarrhea e.g loperamide

☐ Constipation e.g. movicol or lactulose

☐ None

☐ Other

Any other comments you would like to add? (Leave blank if you have nothing to add).

2.18 Anon questionnaire

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Mucositis and infection reduction with probiotics in children with cancer: a randomised-controlled feasibility study protocol

Why did you choose not to participate in the MaCROS study?

What could have been done differently to make you reconsider participation?

Any other comments/suggestions

2.19 Interview template

Interview topic guide

Objectives

Primary aim

1. Explore the participants or parents/guardians experience of participating in the MaCROS study

2. Explore suggestions from participants or their parents/guardians to improve user experience in future studies.

Sample size

Up to 10 participants will be identified and invited to participate. The interview will be done using a telephone or face to face using a semi-structured interview format.

Selection criteria

English speaking

Be a participant/parent/guardian randomised in the MaCROS study OR willing to participate in an interview despite choosing to decline participation.

Interview questions

Recruitment

1.1. What involvement did you have in the MaCROS study?

1.2. Why did you agree to participate?

1.3. Why did you choose to decline participation?

1.4. How did you find the experience of being approached to participate in the MaCROS study?

Randomisation

1.5 How did you find the randomisation process and receiving the probiotic/placebo?

Taking the probiotic/placebo

1.6 How easy or difficult was it to take the probiotic/placebo?

1.7 Did you manage to take it every day?

1.8 If not why?

1.9 How did you or your child find the taste of the probiotic/placebo?

Use of the web-app/paper diary

1.10 Did you choose to use the web-app or paper diary?

1.11. Why did you choose this?

1.12. How did you find the experience of using the web-app/diary?

1.13 What did you like about the diary?

1.14 What didn't you like about the diary?

1.12 Do you have any suggestions to make the diary a better experience for future users?

Communication

1.13 How do you feel about the communication from the The MaCROS research team whilst taking part in the study?

Overall experience

1.14 What did you enjoy about the study?

1.15 What didn't you enjoy about the study

1.16 Do you have any suggestions to improve the experience in a future study?

1.17 Would you recommend this study to another person?

1.18 Is there anything else you would like to mention?

Notes for the Interviewer

Inform the interviewees the session will be recorded. Have a fully charged voice recorder and back up available. The interview can last up until 1 hour.

Open ended questions with topic guides `

It is not necessary to stick to the exact line of questioning or order. The purpose is to be open and explorative. However, it is important, that the subject matters in the topic guides are explored and the style of questioning kept open and explorative.

End of Interview

2.20 The MaCROS study biological substudy

The MaCROS biological sub-study

Introduction

An ongoing randomised-controlled feasibility study investigating the use of liquid probiotics to reduce or prevent mucositis and infection in children with cancer (the MaCROS study) is currently open at Leeds Teaching Hospital Trust (ClinicalTrials.gov Identifier: NCT03785938 (active), IRAS PROJECT ID: 246313). It would also be beneficial to assess the feasibility of undertaking a parallel biological sub-study to investigate the mechanism of action (or lack of) of probiotics in children with febrile mucositis. Therefore, an amendment to the MaCROS study is proposed to include a parallel biological sub-study investigating the mechanism of probiotic response alongside a future randomised-controlled trial.

Background

Mucositis

Mucositis is the inflammation and ulceration of the gastrointestinal mucosal lining which can occur in children diagnosed with cancer. It may be caused by radiotherapy or cytotoxic agents which affect DNA synthesis (particularly S-phase specific agents) including cytarabine, methotrexate and fluorouracil(160).

Mucositis can occur in any part of gastrointestinal system from the mouth to the anus. Symptoms range from mild erythema to wide spread ulceration. Development of mucositis can therefore result in pain, nausea, malabsorption, malnutrition, diarrhoea and increased risk of local and systemic infections(161)

Taking biopsies of the gastrointestinal mucosal lining is the gold standard test to confirm mucositis. However, children typically require an endoscopy under general anaesthesia for this investigation. This is associated with significant risk, particularly as children are immunosuppressed and susceptible to complications including severe infections and bleeding. Because of this, children with suspected mucositis are diagnosed clinically and graded according to the severity of reported symptoms using validated assessment scales such as the National Cancer Institute Common Terminology Criteria for Adverse Events (NCI CTCAE).

The Sonis hypothesis proposes 5 stages of the pathogenesis of mucositis:

Radiotherapy or cytoxic exposure to the mucosal lining resulting in DNA damage and release of free radicals

Activation of transcription factors including NF-kb, which results in the upregulation of proinflammatory cytokines causing mucosal destruction

Signal amplification which may exacerbate or prolong mucosal injury

Ulceration

Healing, and gradual restoration of the flora (162).

The relationship between mucositis and febrile neutropenia is recognised and the term 'febrile mucositis' has been proposed (161). Patients with mucositis are believed to be most vulnerable to bacterial translocation from the gastrointestinal tract during stage 4 of the Sonis hypothesis(140), following damage to the epithelial lining of the mucosa and inflammatory amplification (140).

A study of adult patients diagnosed with multiple myeloma or non-hodgkins lymphoma demonstrated that there was a higher incidence of fever with severe mucositis when compared to those with less severe or no mucositis (68% vs 47%, difference 21%, p=0.004) (161). However, studies investigating mucositis and infection in paediatric cancer patients are limited. A review of guidelines investigated the management of oral mucositis in children undergoing stem cell transplantation highlighted that the epidemiology of mucositis is poorly understood, and that further observational studies and consensus- based approaches are required to understand and develop appropriate risk stratification tools. It also reported that further studies are needed to investigate preventative strategies(161). Currently, management of mucositis involves supportive care strategies. This may include the use of analgesia, loperamide to reduce diarrhoea, and delivery of nutrition using both enteral and parental routes. There are no widespread preventative or therapeutic intervention for febrile mucositis. Recent studies have explored the role of probiotics as a preventative and therapeutic intervention for people with cancer and mucositis.

Probiotics and gastro-intestinal bacterial colonisation

Probiotics are defined as "live micro-organisms which, when administered in adequate amounts, confer a health benefit on the host" according to the World Health Organisation and United Nations Food and Agriculture organization (FAO) (37).

A recent systematic review and meta-analysis investigated the use of probiotics in people with cancer(163). Twenty one studies (N = 2,982 participants) were identified

for assessment of efficacy. Results showed probiotics may reduce the incidence of diarrhoea in patients with cancer [odds ratio (OR) = 0.52, 95% confidence interval (CI) 0.34-0.78, 95% percentage prediction interval (PI) 0.3-0.92, I-sq 36.9%, 5 studies], duration of pyrexia [standardized mean difference 0.64 days, 95% CI 053-0.77, PI 0.64-0.64, I-sq 0.01%, 5 studies] and possibly the severity of diarrhoea [for example Common Toxicity Criteria grade 3 and 4 diarrhoea [OR=0.51, 95% CI 0.12-2.2, PI 0.03-9.08, I-sq 92.5%, 4 studies].

A lack of paediatric patients, heterogeneity of study characteristics and unclear risk of bias reported in included studies highlighted the uncertainty of confidence from conclusions drawn. This review demonstrated that there were insufficient studies to assess the true effect of probiotics in people with cancer. Meta-analysis suggested that probiotics may be beneficial but further studies are still required, particularly in children.

This has led to the development of a randomised-controlled feasibility study to investigate the use of liquid probiotics to reduce or prevent mucositis and infection in children with cancer (the MaCROS study) at Leeds Teaching Hospital Trust (ClinicalTrials.gov Identifier: NCT03785938 (active), IRAS PROJECT ID: 246313).

Further review of the literature identified a paucity of information exploring the biological evidence for the use of probiotics in people with cancer. The systematic review and meta-analysis previously discussed (163) did not undertake a review of the bacterial composition in stool sample of included studies because of the lack of data reported. Investigating the bacterial composition of stool samples would allow further exploration of how probiotics may impact upon bacterial diversity. Assessment of the microbiome, the gut flora, and any colonisation by probiotics observed may help in understanding the interactions between probiotic consumption and mucositis, be it beneficial or ineffective

Using a ‘biological tool’ to explore the severity of mucositis with the use of probiotics could enhance understanding of the etiology, stratification and treatment of mucositis. Therefore, biomarkers may be a useful aid to investigate how probiotics may impact mucositis.

Biomarkers

Biomarkers are defined as “human or animal biological property whose in vitro measurement or identification is useful for the prevention, diagnosis, prognosis, treatment and follow-up of humans or animal diseases, and for their understanding(138)”.

A review of the literature highlighted that biomarkers have been used to identify or stratify the risk of mucositis in adults(139, 140) .Ten biomarkers have been investigated in 4 paediatric studies. These studies reported that:

Serum citrulline may be used to determine the severity of mucositis

Faecal calprotectin maybe used as a non-invasive biomarker for those with mucositis without neutropenia

Serum procalcitonin may be able to distinguish fevers due to bacteraemia from those with mucositis who are febrile due to a systemic inflammatory response

The C-Sucrose breath test is feasible to use in children with cancer

Whilst serum IL-8 is a potential biomarker in children with febrile neutropenia it may not be accurate for use in those who also have mucositis.

The four trials were reported as prospective studies by research authors (including one randomised-controlled trial), however two of these studies reported on a subgroup of participants, of which samples were analysed retrospectively.

All studies had small sample sizes and reporting of the studies were unclear. Significant biases were found in these studies including selection bias (Tooley KL et al), confounding bias (all studies) and outcome information bias (all studies). Reporting of statistical results did not include 95% confidence intervals.

Studies used different definitions and grading tools for mucositis. Gosselin KB et al and Tooley KL focused on oral mucositis only, WJFM van der Velden et al included those with oral and gastrointestinal mucositis (using different grading tools), whilst KG Miedema et al did not make any reference to oral mucositis. It is unclear from these studies whether oral, gastrointestinal or combined mucositis would impact the interpretation of biomarkers.

This review has highlighted the need for further robust studies to explore how biomarkers can be used to investigate the response probiotics may have on mucositis. Faecal calprotectin is a non-invasive biomarker with a relatively low cost to undertake analysis. One stool sample can be used to simultaneously analyse bacterial composition and biomarkers. This is less invasive and more convenient for participants then requiring to attend hospital for a blood test.

Therefore, an amendment to the MaCROS study to include a biological sub-study is proposed. This will investigate the feasibility of testing stool samples for probiotic bacterial colonisation, and faecal calprotectin as a biomarker to explore the effect probiotic consumption in children with mucositis.

Aims and Objectives

Aim

The aim of this biological sub-study is to

Evaluate the feasibility of testing stool samples for probiotic bacterial colonisation

Evaluate the feasibility of using faecal calprotectin as a biomarker explore the effect probiotics may have on mucositis

Objectives

The primary objectives of this sub-study will determine feasibility by recording:

The proportion of participants who agree to participate in the study

The proportion of participants complete the sub-study, including the completion rate of the stool samples returned prior to or on day 0 and days 7, 14, 21 and 28 days

Preliminary health economic information surrounding the costs/benefits of the using biomarkers.

Secondary objectives will include:

Evaluation of the research sub-study protocol

Barriers to compliance with the sub-study protocol

Evaluation of intended outcomes to be assessed if an RCT substudy is undertaken. If sufficient data is available the correlation between faecal calprotectin and severity of mucositis (according to NCI CTCAE grading), mean differences of results between the liquid probiotic and placebo group and reporting of colonisation of probiotic bacteria in stool samples

Sub-study design

Trial design

This will be a biological substudy of a single-centre double-blind randomised-controlled feasibility study.

Study setting

This study will take place at Leeds Teaching Hospital Trust, Leeds UK.

Recruitment

Participants will be recruited from the paediatric haematology and oncology inpatient and outpatient departments at Leeds Teaching Hospital Trust, Leeds, UK.

Participants will be invited to participate in the substudy when they consent to participate in the MaCROS study.

Target recruitment

The recruitment target is up to 10 patients. Recruitment will continue until the MaCROS study closes. As this is a feasibility study, a power calculation is not required.

Eligibility criteria for participants

Participants must be eligible and have agreed to participate in the MaCROS study.

Exclusion criteria

Patients who have already started the course of chemotherapy. Participants who have received IV antibiotics within 7 days of starting their course of chemotherapy.

Methodology

Participants will be randomised in the main study to take the liquid probiotic (Symprove) or placebo on the first day of their chemotherapy/pre-stem cell transplant chemotherapy conditioning and take this once daily for 14 days. The dose will be adjusted according to age.

Additionally, participants will be asked to supply a stool sample prior to or on day 0 and days 7, 14, 21 and 28 days which will be analysed for probiotic bacterial colonisation (Lactobacillus rhamnosus, Lactobacillus plantarum, Lactobacillus acidophilus, and Enterococcus faecium) and faecal calprotectin.

Data collection

Stool samples will be collected in aliquots and transported to the LTHT microbiology laboratory at room temperature within 3 days following collection.

Participants will be given the option to either submit samples to member of the clinical care team for transport or post samples in stamped and addressed packaging that complies with the Royal Mail packaging instruction 650 (e.g. the Safebox product).

Samples will then be stored between −20°C and −70°C in the microbiology laboratory prior to analysis. Results will be issued to the research team (who are also clinicians) within 14 days of processing samples.

Data analysis

Data collected will be confidential according to NHS and University of Leeds data protection regulations. Descriptive statistics (such as probiotic bacterial colonisation and reported faecal calprotectin level from stool samples taken prior to or day 0, and days 7,14,21 and 28) will be reported due to the small of number of participants targeted. If enough data is available outcomes investigated will also include:

The correlation of faecal calprotectin and severity of mucositis (according to NCI CTCAE grading)

Simple descriptive statistical or quantitative analysis

The feasibility of undertaking a biological sub-study will be evaluated using both quantitative and qualitative methods. Data relating to timing of stools submitted, identification of eligible participants, recruitment rate and participants who discontinue the sub-study will be recorded. Acceptability and tolerability of the intervention will be assessed through completion rate of the delivery of stool samples on prior to or on day 0 and days 7 , 14 , 21 and 28 will be explored. Exploration of the patients/parents study participation via interview will be explored as discussed in the MaCROS study protocol. A 'traffic light system' will be used to evaluate the biological sub-study (Table 1).

Indications to consider stopping the sub-study

The occurrence of an unexpected serious adverse event attributed to the biological sub-study. E.g. leakage of stool in transit.

Indications to continue the study without modification

No issues implementing study protocol

Adequate number of participants identified and recruited during the MaCROS study

100% compliance with the delivery of stool samples

No problems undertaking bacterial analysis and faecal calprotectin of stool samples.

Indications to not undertake this biological sub-study within an RCT

Unable to recruit a minimum of 5 participants once the MaCROS study has closed

The occurrence of an unexpected serious adverse event attributed to the biological sub-study. e.g. leakage of stool in transit.

Serious concerns identified when undertaking bacterial composition analysis and measuring faecal calprotectin of stool

Serious concerns identified during qualitative analysis of participants/parents interview.

Table 1: Table summarising the traffic light system used to evaluate the biological sub-study

To not proceed	Modify	Proceed
Less than 5 participants recruited	Difficulty following the protocol e.g. identifying eligible participants	No issues implementing protocol
Occurrence of unexpected serious adverse event	Poor compliance with the delivery of stool samples	Adequate recruitment
Serious concerns identified with faecal bacterial and calprotectin analysis	Concerns identified with the faecal bacterial and calprotectin analysis	100% compliance with the delivery of stool samples
Serious concerns identified during qualitative analysis of participants/parents	Concerns identified during qualitative analysis of participants/parents	No concerns undertaking bacterial analysis and faecal calprotectin levels of stool samples

Dissemination

The results of this feasibility biological sub-study will inform the planning of a definitive biological study alongside the development of a randomised-controlled trial within the evaluatiopn of the MaCROS study. Quantitive and qualitative results will be used to further refine the protocol for biological sub-study to be undertaken alongside large RCT. Findings will be presented at relevant meetings as well as manuscripts submitted to peer-reviewed journals according to the CONSORT extension [10].

2.21 Liability letter from sponsors (University of Leeds)

HENDERSON
INSURANCE BROKERS
an Aon company

Thornton House
Capital Park
Leeds
West Yorkshire
LS27 0TS

T 0113 393 6300

www.hibl.co.uk

17 September 2018

To Whom It May Concern

Dear Sirs,

EVIDENCE OF INSURANCE – The University of Leeds &/or Subsidiary Companies

We are writing to confirm that we act as Insurance Brokers to the above client and that we have arranged liability insurance on their behalf as detailed below:

EMPLOYERS LIABILITY
Cover in respect of indemnity for claims made for death, injury or disease to any person arising out of and in the course of their employment.

INSURER	:	Zurich Municipal
POLICY NUMBER	:	NHE-03CA02-0016
PERIOD OF INSURANCE	:	29th September 2018 – 28th September 2019
LIMIT OF INDEMNITY	:	£40,000,000 each occurrence including costs and expenses

PUBLIC/PRODUCTS LIABILITY
Indemnity in respect of claims made for death, injury or disease to persons (other than employees) or loss or damage to third party property arising out of and in the course of the business.

INSURER	:	Zurich Municipal
POLICY NUMBER	:	NHE-03CA02-0016
PERIOD OF INSURANCE	:	29th September 2018 – 28th September 2019
LIMIT OF INDEMNITY	:	£40,000,000 each occurrence (and in the aggregate in respect of Products)

PROFESSIONAL INDEMNITY
Indemnity in respect of the Legal Liability to Third Parties for breach of professional duty due to negligent act, error or omission in connection with your business.

INSURER	:	Royal & Sun Alliance
POLICY NUMBER	:	RKI0855002
PERIOD OF INSURANCE	:	29th September 2018 – 28th September 2019
LIMIT OF INDEMNITY	:	£10,000,000 each occurrence and in the aggregate

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/Continued.....

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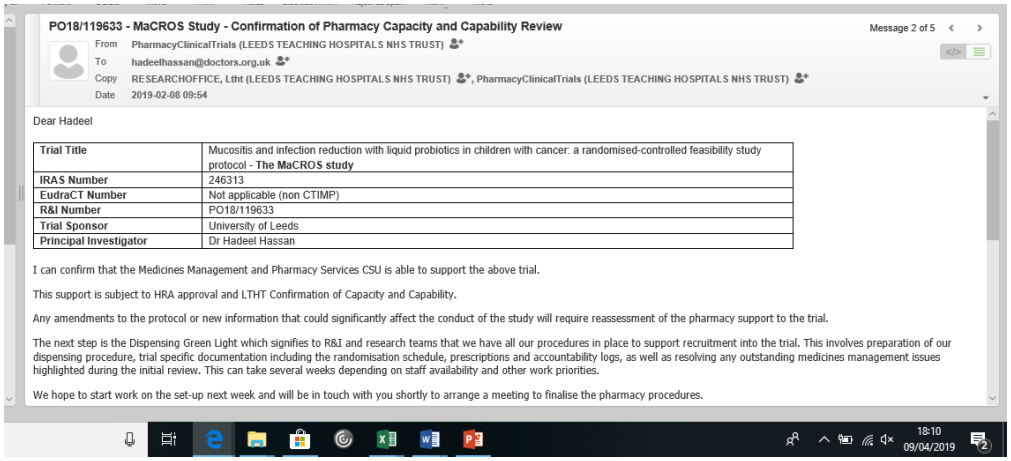
If you should require any further information or the above please do not hesitate to contact us.

Yours faithfully,

Rhydian Thomas
Director

Direct Dial: **0113 393 6826**
Email: rhydian.thomas@hibl.co.uk

2.22 Confirmation of Pharmacy Capability approval



PO18/119633 - MaCROS Study - Confirmation of Pharmacy Capacity and Capability Review

Message 2 of 5

From PharmacyClinicalTrials (LEEDS TEACHING HOSPITALS NHS TRUST)
To hadeelhassan@doctors.org.uk
Copy RESEARCHOFFICE, Ltht (LEEDS TEACHING HOSPITALS NHS TRUST), PharmacyClinicalTrials (LEEDS TEACHING HOSPITALS NHS TRUST)
Date 2019-02-08 09:54

Dear Hadeel

Trial Title	Mucositis and infection reduction with liquid probiotics in children with cancer: a randomised-controlled feasibility study protocol - **The MaCROS study**
IRAS Number	246313
EudraCT Number	Not applicable (non CTIMP)
R&I Number	PO18/119633
Trial Sponsor	University of Leeds
Principal Investigator	Dr Hadeel Hassan

I can confirm that the Medicines Management and Pharmacy Services CSU is able to support the above trial.

This support is subject to HRA approval and LTHT Confirmation of Capacity and Capability.

Any amendments to the protocol or new information that could significantly affect the conduct of the study will require reassessment of the pharmacy support to the trial.

The next step is the Dispensing Green Light which signifies to R&I and research teams that we have all our procedures in place to support recruitment into the trial. This involves preparation of our dispensing procedure, trial specific documentation including the randomisation schedule, prescriptions and accountability logs, as well as resolving any outstanding medicines management issues highlighted during the initial review. This can take several weeks depending on staff availability and other work priorities.

We hope to start work on the set-up next week and will be in touch with you shortly to arrange a meeting to finalise the pharmacy procedures.

18:10
09/04/2019

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