RESEARCH PROJECT PROTOCOL

A retrospective cross-sectional cohort trial assessing the implications of MTHFR polymorphisms on DNA methylation and platinum resistance in ovarian cancer patients

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Co-Investigator: Dr Michael Watson (PhD)

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| Title | A retrospective cross-sectional cohort trial assessing the implications of MTHFR polymorphisms on DNA methylation and platinum resistance in ovarian cancer patients |
| Purpose | To assess the influence of methylene tetrahydrofolate reductase (MTHFR) polymorphisms and diet on platinum responsiveness in ovarian cancer patients |
| Aims | 1. How might platinum response be affected by the presence of MTHFR polymorphisms. 2. What role does dietary and/or supplementary intake of folate play in platinum resistance. 3. Can we identify a contributor or protective prognostic marker in platinum resistance. |
| Design | Retrospective, cross-sectional cohort (pilot) study |
| Number of Participants | The total number of participants in the trial will be n=30 based on the central limit theory, with a power of 48% and confidence level of 95% |
| Types of Participants | Adult females aged 18 – 80 years |
| Key Inclusion Criteria | * Diagnosed with ovarian, fallopian tube, epithelial or peritoneal cancer * Completed first line platinum therapy * Diagnosed with refractory/resistant disease or platinum sensitive disease a minimum of 6 months after platinum therapy |
| Key Exclusion Criteria | * Non-English speaking * Never received a platinum drug for ovarian cancer diagnosis |
| Duration | The trial will run for 8 weeks. There will be a single data collection point for each participant. |
| Outcome measures | ***Primary Outcome* will measure;**     * To examine any differences between MTHFR polymorphism presence in platinum resistant versus platinum sensitive patients with ovarian cancer.   ***Secondary Outcomes* will measure;**   1. To investigate folate intake (epigenetic or direct impact on cancer progression) and difference between platinum resistant and platinum sensitive ovarian cancer patients. 2. Investigate any correlations or predictors of platinum resistance or sensitivity in ovarian cancer patients which may be related to nutrient intake and/or MTHFR status |
| Study centre / site | Multi-centre trial located at ICON Cancer Care Southport and Brisbane Day Hospitals |
| Ethics Approval | Ethics approval will be through ICON Cancer Care HREC and Endeavour College of Natural Health HREC.  The trial will be registered on ANZCTR |
| Data Analysis | All data will be analyzed on STATA ©14. Associations between variables such as the presence or absence of MTHFR polymorphisms will be subject to the chi-square test. Logistic regression will be applied to determine the correlation between the existence and/or absence of any of the test variables (folate intake and/or MTHFR polymorphisms) and platinum response. Further analysis may occur once all variables are determined. |

**List of Abbreviations**

CPG island Cytosine phosphate guanine island

CRF Case Report Form

DQES v3.2 Dietary Qestionnaire version 3.2

FACT-O Function Assessment of Cancer Therapy – Ovarian Cancer

MTHFR Methylene Tetrahydrofolate Reductase

SNP Sullivan and Nicolaides Pathology

UIC Unique Identifier Code

# Preface

This document is a clinical research protocol for a human research study. This study is to be conducted according to the Australian Clinical Trials Guidelines: Australian Government, National Health and Medical Research Council, Department of Industry, Innovation and Science.

# Aim

The research project aims to investigate three significant questions relating to platinum resistance and ovarian cancer.

1. How might platinum response be affected by the presence of MTHFR polymorphisms.
2. What role does dietary and/or supplementary intake of folate play in platinum resistance.
3. Can we identify a contributor or protective prognostic marker in platinum resistance.

### 1.1 **The hypothesis**:

The presence of MTHFR polymorphisms may improve response to platinum drug therapy in ovarian cancer.

### 1.2 **The objectives:**

1. To examine whether there is a difference between MTHFR polymorphisms in platinum resistant versus platinum sensitive patients with ovarian cancer.
2. To investigate folate intake (epigenetic or direct impact on cancer progression) and the difference between platinum resistant and sensitive patients with ovarian cancer
3. To investigate any correlations or predictors of platinum resistance or sensitivity in ovarian cancer patients which may be related to nutrient intake and/or MTHFR status.

Research regarding platinum resistance or sensitivity and biomarkers indicates that this area has not been investigated to date.

# **Background**

### **2.1 Ovarian Cancer**

Ovarian cancer is the leading cause of gynecological cancer death world-wide. Highly curable if diagnosed in its early stages, 70% of cases are not diagnosed until the cancer has reached advanced stage (Koukoura, Spandidos, Daponte, & Sifakis, 2014). The first-line treatment for this cancer is chemotherapy with a platinum and taxane agent (Hennessy, Coleman, & Markman, 2009). The antitumour activity of platinum compounds is dependent on the platination of DNA strands, causing intra- and interstrand breaks, leading to p53-initiated apoptosis (Cepeda et al., 2007). Certain patients have been found to be resistant to platinum therapies and prognostic biomarkers may be predictive of chemotherapy response. It is also predicted that up to 20% of all ovarian cancer patients are intrinsically resistant to platinum-based therapies significantly increasing toxicities and reducing overall survival (Assis et al., 2017; Balch, Fang, Matei, H-M Huang, & Nephew, 2009). Platinum resistance poses a significant challenge to the clinical approach in affectively treating ovarian cancer patients. Since platinum-therapy inception very little advancement has been made in combating resistance in a population where 5-year overall survival is as low as 30% (Gifford, Paul, Vasey, Kaye, & Brown, 2004).

### **2.2 MTHFR, Folic Acid and DNA methylation**

It is estimated that up to 70% of the world’s population has one or more alleles affected by polymorphisms in the MTHFR enzyme, resulting in a reduction of enzyme activity, thereby impacting folate utilisation and conversion, homocysteine and methionine recirculation and potentially cellular antioxidant status, DNA synthesis and methylation specific epigenetic changes in cellular activity (Long & Goldblatt, 2016).

Methylene tetrahydrofolate is an essential contributor to DNA synthesis (Rosenberg et al., 2002). Likewise, folic acid is crucial for genome stability and preventing the hypomethylation of DNA (Fenech, 2001). MTHFR enzyme is essential in the conversion of dietary and supplemental folates into active folate for use in one-carbon transfer reactions associated with the methylation of homocysteine to methionine, DNA replication, neurotransmitter synthesis and intracellular antioxidant status (Gropper & Smith 2013, pp. 344 – 350).

In 2009 it became mandatory in Australia, for foods such as breads and cereals, to be fortified with a synthetic form of folate, folic acid to aid in the reduction of neural tube defects in children (Crider, Bailey, & Berry, 2011, p. 377). From a holistic perspective, it is well understood that isolated vitamins and minerals may lack the synergistic benefit that whole food forms provide in the body. However, from a biomedical perspective, the synergistic effects of foods are only now being considered. The impact of these isolated forms compared to synergistic nutrients on human health is yet to be completely understood (Jacobs Jr., 2014; Tapsell, Neale, Satija, & Hu, 2016, p. 445).

Low folate status has been implicated in the development of some cancers, however current research identifying tumour folate receptors suggest that whilst folate status may play a protective role against cancer in healthy populations, unmetabolised folic acid may be associated with the promotion of cancer cell growth and drug resistance (Baldauff, 2013; Cho et al., 2015; Koukoura et al., 2014, p. 6; Sauer, Mason, & Choi, 2009, p. 5).

Furthermore, ovarian tumours contain increased intracellular levels of glutathione (GSH) a potent antioxidant, that may play a role in the resistance of platinum drug therapy and is reliant on the availability of amino acids cysteine, glycine and glutamate (Verschoor & Singh, 2013, p. 141). GSH is recycled through the methionine pathway by the folate dependent methylation cycle.

It is currently unclear whether DNA methylation among humans can be altered by folate intake, or whether the patterns in methylation change, dependant on the timing and extent of exposure (Crider et al., 2011).

### **2.3 Literature Review**

A review of the current literature revealed a large number of biomarkers related to platinum therapy response in ovarian cancer patients and that some of these biomarkers may be used as prognostic indicators. It is further revealed that methylation and histone modifications may play a role in the association with biomarkers, such as BRCA1, hMLH1 and LINE-1, chemoresponse and subsequent clinical outcome (Koukoura et al., 2014). Moreover, epigenetic changes may also contribute to the relationship between biomarkers and chemoresponse rate, as well as variability in individual responses to therapy (Balch et al., 2009).

Epigenetic regulation of DNA in cancer includes DNA methylation of CpG islands and histone modifications, changes in genetic behaviour without altering the sequence of DNA (Rodríguez-Paredes & Esteller, 2011). Since genetic alterations are nearly impossible to correct and cannot alone account for the complexities of platinum resistance (Koukoura et al., 2014), epigenetic modifications in cancer makes them the target for prevention and/or treatment strategies.

# **Methodology**

### **Study design**

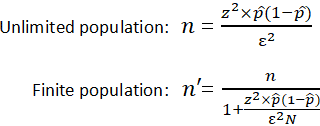
The research project is a retrospective cross-sectional cohort observational trial. Participants will be selected based on ovarian cancer diagnosis and response to chemotherapy, placed in one of two groups, platinum resistant or platinum sensitive. Platinum refractory disease patients, whose tumours continued to grow throughout primary therapy will be included in the platinum resistant group. The two groups will be compared for the presence or absence of MTHFR polymorphisms, dietary and supplementary intake of folate prior to diagnosis and during chemotherapy and serum active B12, homocysteine, and serum folate levels in a one-off blood test conducted by SNP.

### **3.2 Participants**

The target population for this study will be female adults aged 18 to 80 years old and meet the inclusion criteria as outlined below. Participation in this clinical trial is sought to identify possible contributors and/or biomarker for platinum drug response in ovarian cancer patients.

**3.3 Sample size**

By selecting a sample size of 30 participants for the study with a 95% confidence level, this would reflect a CI of 17.89%. In the event that 70% of participants have an MTHFR mutation, as has been suggested by world-wide population estimates, the investigators can be confident that between 52.11% and 87.89% of the sample will contain an accurate reflection of possibility, equating to between 16 and 26 participants showing some correlation (Figure 1).



**Figure 1. Sample Size Calculation:**  n= sample size, z score is based on confidence level (being ±1.96 based on 95%), p= population proportion and N= population size (Kadam & Bhalerao, 2010).

### **3.4 Inclusion and Exclusion Criteria**

#### 3.4.1 Inclusion criteria

* Diagnosed with ovarian, fallopian tube, epithelial or peritoneal cancer
* Completed first line platinum therapy
* Diagnosed with refractory/resistant disease or platinum sensitive disease.

#### 3.4.2 Exclusion criteria

* Non-English speaking
* Never received platinum drug therapy for ovarian cancer

### **3.5 Recruitment**

Recruitment of participants will take place through day hospital units located on the Gold Coast and Brisbane, Australia through day oncology hospitals belonging to ICON Cancer Care. Recruitment will be supervised by medical oncologist, Dr. Jim Coward (BSc(Honours) MBBS MRCP (UK) FRACP PhD). Prospective participants will be identified using ICON’s patient database and will be notified of the trial by mail and on-site. An information session outlining the trial will be provided to all interested participants and an information and consent form provided for perusal. Consent will be sought to access medical records, the provision of a single blood test and the use of blood serum for future use in this trial if necessary (Appendix A). Participants will be deidentified using a UIC generated by Totalcare© software.

### **3.6 Consent and Procedures**

#### 3.6.1 Pre-trial

The formal consent will be signed after the following criteria have been met; the researcher is satisfied the participant has an individual understanding of the trial, and the Patient Information Sheet has been read thoroughly by the participant and any supporting family members if the participant requests their attendance.

A consent form for the participant to sign together with the Researcher’s signature is to be kept by the participant as their copy of the consent. A second consent signed by both the participant and the Researcher is the Researcher’s copy of the consent and is to be filed in a locked filing cabinet, in the Researcher’s locked office. An electronic copy of both these consent forms will be generated into a PDF format and stored electronically with password protected signature to maintain confidentiality and anonymity of the participant.

#### 3.6.2 Setting

The research setting is ICON Cancer Care Day Hospital Southport, Level 9, 39 White Street Southport, Gold Coast and The Mater Medical Centre (ICON Cancer Care South Brisbane), level 5, 293 Vulture Street, South Brisbane. ICON research committee have verbally agreed to a reciprocal ethics agreement between Endeavour College of Natural Health and ICON Cancer Care. A hardcopy of this protocol will be provided to ICON Cancer Care ethics committee once available.

### **3.7 Data collection**

### *3.7.1 MTHFR Polymorphisms*

MTHFR analysis will be based on three criteria:

• Presence of mutation – positive or negative

• SNP (single nucleotide polymorphism) variation – heterozygous or homozygous

• Call letter and location variation – A/C/T

Collection will be performed by trained nurse collection staff at SNP and analysed as per SNP procedures and protocols by their pathologists.

### *3.7.2 Instruments*

There will be two instruments that will be used in this trial for quality of life, the FACT-O (Functional Assessment of Cancer Therapy – Ovarian Cancer)(Appendix B) and nutrient intake assessment, DQES v.3 (dietary questionnaire version 3.2)(Appendix C). Permission has been granted from the authors for the use of the FACT-O (Appendix D) and the DQES v3.2 will be purchased through the Cancer Council Victoria. The participant will complete the FACT-O and DQES v3.2 at the single data collection point, to assess the impact of their chemotherapy regime on quality of life, and their nutrient intake throughout the past 12 months of treatment.

### *3.7.3 Medical Records*

The extraction of key data from medical records is an essential factor in the study. Collecting data on age, tumour stage and grade at diagnosis, the presence of metastasis, surgical history including the presence of residual disease at the commencement of chemotherapy, time to relapse, family and subsequent genetic history, and existing comorbidities and medication history. All patient data will be collected using an extensive CRF allowing for data to be confidentially and thoroughly compiled without the risk of a data breach resulting in sensitive information being leaked or accessed by any external parties (Appendix E). CRF’s will be identifiable only through the UIC.

### **3.8 Data management**

All participant hard copy files which include; CRF, PISCF, DQES v3.2 and FACT-O will be kept in individual files in plastic sleeves in the participants medical file located in the medical records department. This is inside the locked ICON Cancer Care Southport Day Hospital, which is located at Level 9, 39 White Street Southport, Queensland. The only people accessible to this office are the staff and doctors employed by ICON Cancer Care. Electronic forms and logs will be maintained as password protected files on the computers of the primary investigator (Caitlin Phillips-Chavez) which are only accessible by the researcher named. These password protected computers are in a locked officewithin ICON Cancer Care Southport.

The hard copy data files will be stored in a locked medical records department of ICON Cancer Care Southport Day Hospital for fifteen (15) years, then destroyed permanently.

### **3.9 Withdrawal**

Participants are free to withdraw from the trial at any point without question or penalty. This is verbalised by the participant when they either contact the researcher via email or phone. There is allocated space on the CRF for the participant to complete the withdrawal of consent. If the parent withdraws consent over phone or email, it will be noted in the CRF, located on the last page of the document.

### **3.10 Data analysis**

All data will be analysed on STATA ©14. All data will be analysed on STATA ©14. Associations between variables such as the presence or absence of MTHFR polymorphisms will be subject to the chi-square test. Logistic regression will be applied to determine the correlation between the existence and/or absence of any of the test variables (folate intake and/or MTHFR polymorphisms) and platinum response. Further analysis may occur once all variables are determined.

### 3**.11 Outcome Measures**

#### 3.11.1 Primary Outcome Measures

The Primary outcome measure will be to examine any differences between MTHFR polymorphism presence in platinum resistant versus platinum sensitive patients with ovarian cancer.

#### 3.11.2 Secondary Outcome Measures

1 – The secondary outcome measures will investigate folate intake (epigenetic or direct impact on cancer progression) and difference between platinum resistant and platinum sensitive ovarian cancer patients and;

2 - Investigate any correlations or predictors of platinum resistance or sensitivity in ovarian cancer patients which may be related to nutrient intake and/or MTHFR status

Quantitative assessment of the possible impact of folate intake during treatment will be conducted using the DQES\_v3.2® from the Cancer Council Victoria. Case reporting will include supplement use prior to and during diagnosis and chemotherapy, as well as medications that may impact nutrient intake. Validated FFQ have been significantly correlated with serum folate levels (p=<0.01) in previous studies, qualifying their usefulness in appropriately assessing dietary folate intake (Fayet, Flood, Petocz, & Samman, 2011; Pufulete, Emery, Nelson, & Sanders, 2002).

Quality of life will also be assessed using the FACT-O® (FACIT.org, 2017) tool to quantify how treatment has affected the target population and may further substantiate the need for biomarker research to be undertaken in this particularly vulnerable group of resistant patients. Previous research highlights the impact of side effects related to diagnosis and treatment has on well-being and overall functioning (Gupta, Braun, Staren, & Markman, 2013).

### **Limitations**

ICON Cancer Care is a ‘referral centre’ that attracts patients from a higher socioeconomic demographic. It has been reported that the purchasing and consumption of unhealthy diets including the intake of lesser fruits and vegetables, is strongly correlated with socioeconomic status and that higher diet quality is associated with higher cost (Pechey, Monsivais, Ng, & Marteau, 2015; Rehm, Monsivais, & Drewnowski, 2015).

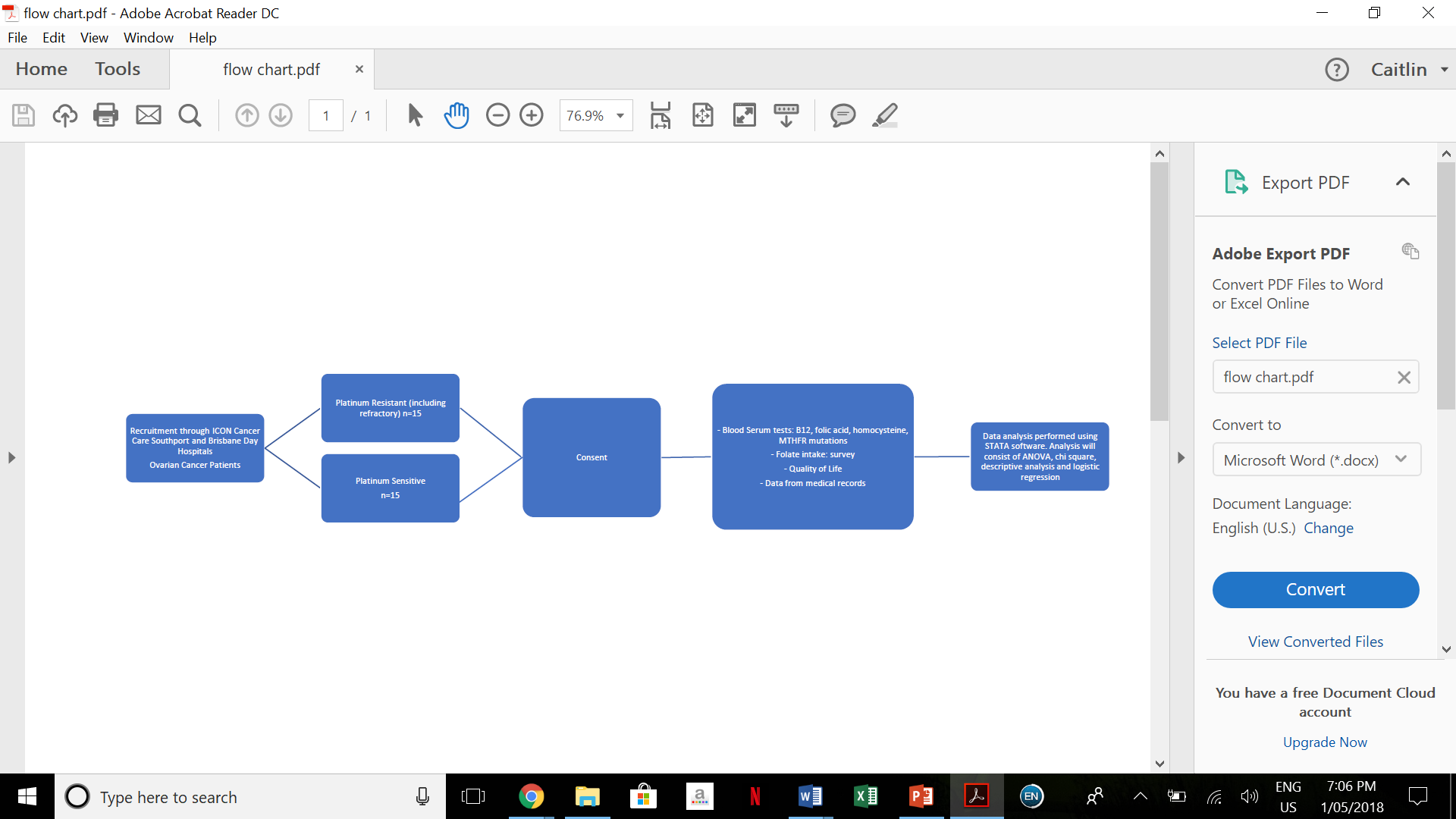
# **Study Procedures**

### *4.1 Participant Attendance*

Participants will be asked to attend an information session run by the Primary investigator on the trial for information and to be provided with consent forms to take home for consideration. Participants will then be asked to return their consent forms to their participating ICON site, where the Primary investigator and oncologist can run through the details of the trial and to clarify the participant’s intent to consent. At the stage of consent, the participant will be provided with their blood test request form, and DQES v3.2 log in details. The Primary investigator will run through the CRF with the participant to capture all the necessary information. The time required of the participant is estimated at one hour to complete the CRF. There is only one point of contact necessary for the participant to attend ICON for the trial which can completed at any point within the trial period, allowing for the required 2 weeks for Cancer Council Victoria to analyse DQES v3.2 data. Blood tests can be actioned onsite on the same day or on any day that is convenient for the participant. Results and analysis of the DQES v3.2 are electronically provided to the Primary investigator as are the blood serum results, which are electronically matched and uploaded into participant files based on their UIC. The FACT-O should be completed at the time of the CRF, however participants can be given a private space and time for its completion if they feel it’s necessary. Information regarding support services can also be provided to the participant if they are feeling distressed by the FACT-O.

### *4.2 Medical Records*

Any information missing from the CRF due to participants not being able to provide it can be retrieved from the participants charts, as per consent. Further data will be collected to satisfy information regarding tumour stage and grade, residual disease, comorbidities or past blood and genetic testing that have been done, from participant medical records, as per consent. All further information will be filled out in the participants corresponding CRF which is identified only by UIC (figure 2).



**Figure 2: Project Plan:** Schematic representation of the project plan from recruitment to data collection and analysis.

# **Finance and Insurance**

### 5.1 Funding Source

Funds will be sourced from the Endeavour College of Natural Health for the Honours project to the worth of $1000. All other funds will be through the Chief investigator and Primary supervisor, Dr Janet Schloss, Endeavour research funds.

### 5.2 Conflict of Interest

All investigators declare no conflict of interest in relation to this study.

### 5.3 Insurance

Clinical trial insurance will be provided by Endeavour College of Natural Health by Mackenzie Ross.

# **Publication Plan**

1. Literature Review: First author, Caitlin Phillips-Chavez
2. Results: First author, Caitlin Phillips-Chavez

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