FULL PROTOCOL TITLE

CONTROL NETs TR: A translational sub-study of CONTROL NETs ("Capecitabine ON Temozolomide Radionuclide therapy Octreotate Lutetium-177 NeuroEndocrine Tumours Study")

STUDY CHAIRS

Dr Ben Lawrence

Prof Nick Pavlakis

Prof Cristin Print

Dr Cherie Blenkiron

A/Prof Chris Hemmings

A/Prof Alex Dobrovic

Dr Sonia Yip

Mr James Murray

Ms Kate Wilson

PROJECT MANAGER / TRIAL COORDINATOR

Evonne Tim

controlnets.study@sydney.edu.au

LINK TO STUDY

https://gicancer.org.au/clinical-trial/control-nets-tr/

TRIAL IDENTIFIER

N/A

COORDINATING CENTRE

University of Auckland

University of Sydney on behalf of the NHMRC Clinical Trials Centre

FUNDING SOURCES

AGITG Innovation Grant 2020

PRESENTER

Dr Ben Lawrence

University of Auckland

FINANCIAL DISCLOSURE

None to declare.

AIM/S

This exploratory sub-study is a multi-omic assessment of MGMT as a biomarker to predict which patients will benefit from the addition of CAPTEM to PRRT, using samples gathered from participants in the CONTROL NETs trial.

BACKGROUND

The AGITG-sponsored, investigator-initiated CONTROL NETs is a clinical trial for the treatment of advanced pancreatic neuroendocrine tumours (pNETs) and midgut neuroendocrine tumours (mNETs). It is a non-comparative randomised open label parallel group phase II trial with 2:1 randomisation to PRRT/CAPTEM (experimental arm) vs. PRRT (mNETs control) and CAPTEM (pNETS control).

This translational sub-study of CONTROL NETs proposes that CAPTEM response is due to a favourable tumour genome, and that specific multi-omic tumour testing of O-6-methylguanine-DNA methyltransferase (MGMT) can predict those who will benefit from this clinical strategy. The cytotoxic DNA damage caused by Temozolomide (TEM) is repaired in normal cells by the enzyme encoded by MGMT, and so lower levels of tumour MGMT are associated with better response to TEM. In some brain tumours MGMT expression is reduced by gene promotor hypermethylation. The methylation test has more sensitivity than testing protein using immunohistochemistry (IHC), which is more difficult to quantitate and subtle changes that might be important in the cell are more difficult to detect. Thus, in glioblastoma, detection of MGMT promotor methylation as a predictive biomarker for TEM has been in clinical use for more than a decade.

MGMT has been tested in NETs; some have reduced expression on IHC, and some have MGMT promotor methylation. Testing MGMT has been predictive for response to TEM in some but not all studies. However, these studies tested mechanisms of MGMT expression extrapolated from brain tumours (e.g., hypermethylation), not from preclinical data in NETs.

Recent genomic studies suggest that promotor hypermethylation is only one of several ways that MGMT expression can be altered in NETs. For example, there is plausible evidence that MGMT expression is reduced by haploinsufficiency in some pancreatic NETs through loss of heterozygosity of chromosome 10 (location of the MGMT gene). Further, MGMT cooperates with a family of genes to repair DNA damage, and changes in expression of related genes through numerous mechanisms could also impact on the effectiveness of TEM. Therefore, accurate predictive testing for TEM in NETs will need to be multi-omic to detect these different forms of MGMT deficiency.

STUDY DESIGN

CONTROL NETs participants consented to the use of their archival pre-treatment tumour samples, prospectively collected blood samples, and for use of these in subsequent genomic analyses.

Since the archived tumour samples were initially collected for clinical purposes, they are of variable quality for genomic analyses. Some samples are small, some have nil or little residual tissue, and the age and fixation is variable; this will affect the yield of nucleic acids, the quality of the sequencing, and number of samples that provide adequate data. MGMT will be assessed by protein expression

(IHC), promotor methylation, and Chromosome 10 ploidy using methods that maximise the utility of these archived samples.

Review of pathology reports when planning the translational substudy suggested that: 39 samples (32 mNET, 7 pNET) are likely to be good quantity and we estimate that 80% of these cases will produce adequate sequencing data (n=31); 29 samples are of uncertain quantity (16 mNET, 13 pNET) and we estimate that 30% of these cases will produce adequate sequencing data (n=9); and 10 samples (5 mNET, 5 pNET) are likely to be exhausted (no tissue left for analysis) and we estimate that none of these cases will produce adequate sequencing data. Therefore, the study design expected that samples from 40 of the 75 participants would be suitable for analysis.

ELIGIBILITY CRITERIA

Samples gathered from the CONTROL NETs trial with remaining tissue of sufficient quality/quantity for the assays.

STUDY UPDATE

SPECIMENS COLLECTED

The CONTROL NETs trial had 75 participants. We received tissue from 68 participants (77 samples), of which there was sufficient tissue for methylation, copy number and IHC analysis in 48 of these participants (52 samples), slightly better than expected.

CHROMOSOME 10 COPY NUMBER ASSAY

We used a shallow whole genome sequencing assay worked up by Prof Print's group to accurately catalogue the copy number status of all chromosomes, including Chromosome 10, where the MGMT gene resides. Of the 44 participants analysed via this copy number assay, we were able to call a Chr 10 copy number for all 44 participants.

MGMT HYPERMETHYLATION ASSAY

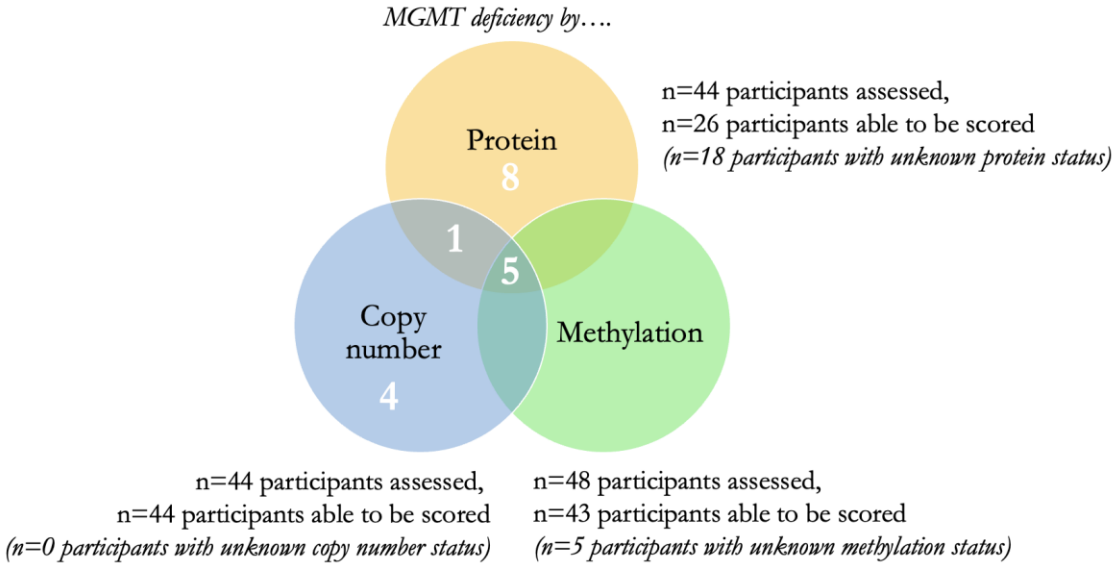
We used a methylation-sensitive high resolution melting point assay developed and run by A/Prof Dobrovic to measure MGMT hypermethylation. Of the 48 participants with sufficient tissue to analyse, we were able to unambiguously score the methylation status of 43/48.

IHC ASSAY FOR PROTEIN EXPRESSION OF MGMT

We used IHC to measure the protein expression of MGMT. IHC was completed by Comparative Biosciences using the MGMT IHC protocol used by the ECOG-ACRIN study (RCT CAPTEM vs TEM). Slides were reviewed by two independent pathologists in the first instance, to indicate whether staining was successful via assessment of positive control tissue, and to assign a H-score indicative of staining intensity. Where there was disagreement, a third independent pathologist also provided scores to reach a consensus. Samples from the 44 participants with sufficient tissue to analyse found 26/44 were able to be scored for high or low MGMT protein expression. The remainder of the samples failed the positive control staining, indicative of the unreliability of this assay, which has also been reported in the literature.

MGMT DEFICIENCY BY ANY OF THREE MEASURES

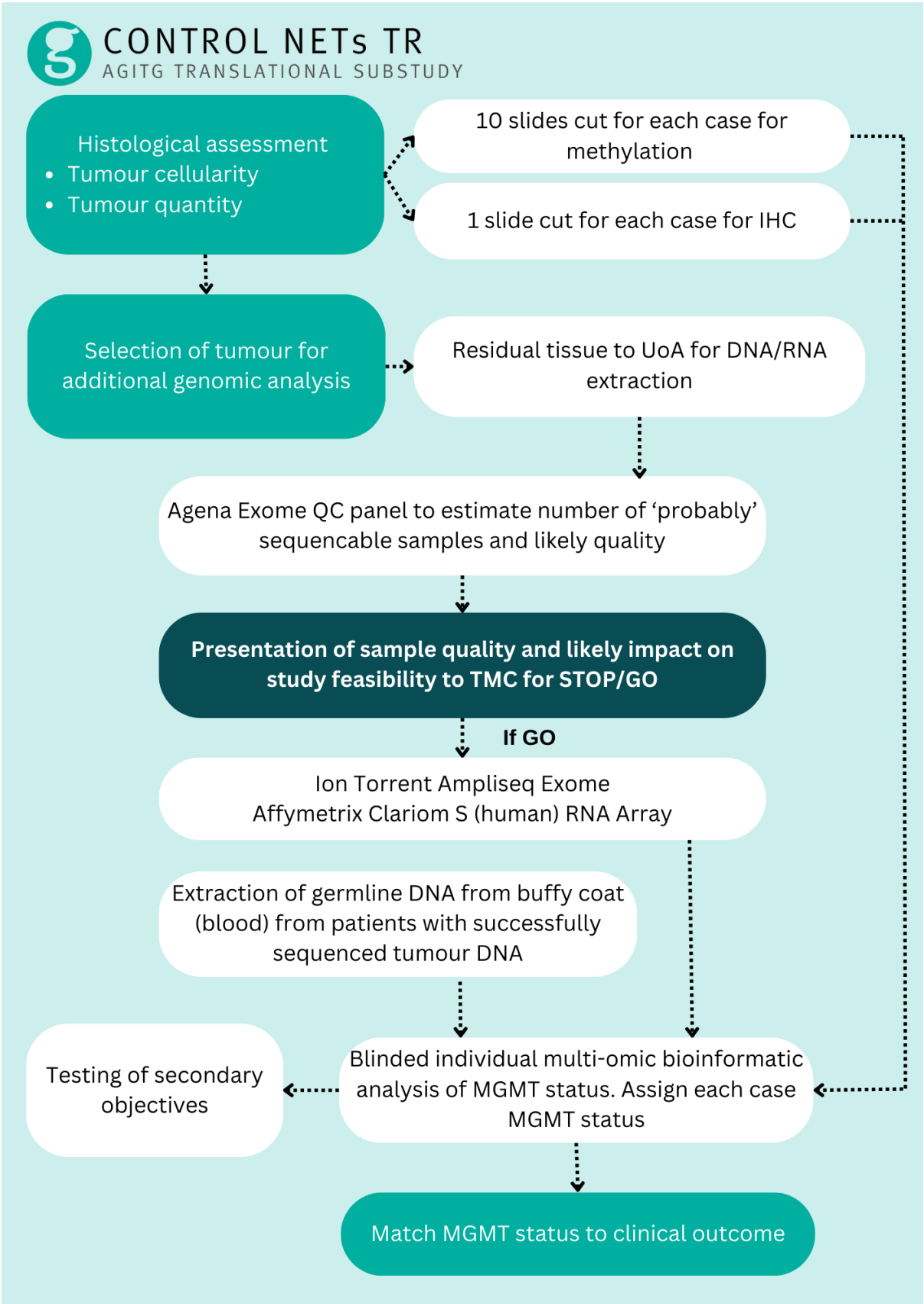
When considering any evidence of MGMT deficiency in the samples from protein, copy number or methylation information, 18/48 participants showed MGMT deficiency (see accompanying figure). Of these 5 showed deficiency via all three measurements. Only 1/52 samples analysed was unable to be scored, due to no information coming from any of the three measurements.



CORRELATION WITH CLINICAL OUTCOME

Now that all of the biological data has been generated, statistical analysis is underway to test the primary objective, that MGMT deficiency by any of these three measurements predicts which patients will benefit from the addition of CAPTEM to PRRT. Of note, 4 IHC samples are still undergoing consensus review by the pathologists, which might alter these numbers slightly.

SCHEMA


CONTROL NETs TR
AGITG TRANSLATIONAL SUBSTUDY
Histological assessment
• Tumour cellularity
• Tumour quantity
10 slides cut for each case for methylation
1 slide cut for each case for IHC
Selection of tumour for additional genomic analysis
Residual tissue to UoA for DNA/RNA extraction
Agena Exome QC panel to estimate number of 'probably' sequencable samples and likely quality
Presentation of sample quality and likely impact on study feasibility to TMC for STOP/GO
If GO
Ion Torrent Ampliseq Exome
Affymetrix Clariom S (human) RNA Array
Extraction of germline DNA from buffy coat (blood) from patients with successfully sequenced tumour DNA
Testing of secondary objectives
Blinded individual multi-omic bioinformatic analysis of MGMT status. Assign each case MGMT status
Match MGMT status to clinical outcome