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First-in-human phase I clinical trial of a combined immune modulatory approach using TetMYB vaccine and Anti-PD-1 antibody in patients with advanced solid cancer including colorectal or adenoid cystic carcinoma: The MYPHISMO study protocol (NCT03287427)

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ABSTRACT

Background: MYB is a transcription factor that is overexpressed in colorectal cancer (CRC) and also a driver mutation in adenoid cystic carcinoma (AdCC). Therefore, the MYB protein is an ideal target to vaccinate against to aid recruitment of tumour infiltrating lymphocytes (TILs) against these tumours. The Peter MacCallum Cancer Centre (Melbourne, Australia) has engineered a DNA vaccine, TetMYB, based on the pVAX1 plasmid vector carrying a fusion construct consisting of the universal tetanus toxin T-cell epitopes flanking an inactivated *MYB* gene.

Methods: This prospective first-in-human phase I single-arm multi-centre clinical trial involves patients with metastatic CRC or AdCC. Stage 1 will evaluate the safety profile of escalating doses of TetMYB vaccine, given sequentially and in combination with an anti-PD-1 inhibitory antibody, to determine the maximum tolerated dose (MTD). Stage 2 will assess the MTD in an expanded cohort. The calculated sample size is 32 patients: 12 in Stage 1 and 20 in Stage 2. The expected total duration of the trial is 3 years with 15 months of recruitment followed by a minimum of 18 months follow-up.

Discussion: MYB transcription factor is aberrantly overexpressed in a range of epithelial cancers, not limited to the above tumour types. Based on promising pre-clinical data of vaccine-induced tumour clearance and establishment of anti-tumour memory, we are embarking on this first-in-human trial. If successful, the results from this trial will allow progression to a Phase II trial and validation of this breakthrough immunotherapeutic approach, not only in CRC and AdCC, but other MYB over-expressing cancers.

Trial registration: ClinicalTrials.gov ID: NCT03287427. Registered: September 19, 2017.

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1. Background

1.1. Colorectal cancer (CRC) and adenoid cystic carcinoma (AdCC)

Colorectal cancer (CRC) is the third most common cancer in the United States with an estimated 135,430 new diagnoses in 2017 [1]. Survival statistics vary with stages of disease, ranging from 89.9% for Stage I to an abysmal 13.9% for Stage IV [1]. It is the second leading cause of cancer related death; in 2017, responsible for approximately 50,260 mortalities [1].

Adenoid cystic carcinoma (AdCC) is a rare malignancy that arises within secretory glands, most commonly the major and minor salivary glands of the head and neck. Other sites include the trachea, lacrimal gland, breast, skin, and vulva. Current management is local resection ± adjuvant radiotherapy. There is no effective systemic therapy for metastatic or unresectable disease.

The *MYB* gene encodes a DNA binding transcription factor that plays an important role in cellular proliferation and differentiation [2]. A connection between the *MYB* gene and cancer has been established over the past decades and has led to the classification of MYB as a *bona fide* oncoprotein. Accumulated studies have shown that *MYB* expression plays an essential role in driving a range of malignancies [2,3]. In malignancies of epithelial nature such as CRC, *MYB* is frequently aberrantly expressed and when expressed at the high range is a property that correlates with poor prognosis for patients with CRC [4]. MYB expression is also more pronounced in secondary CRC tumour sites. The central role of *MYB* in CRC is also observed in other malignancies including breast and pancreatic cancer. AdCC is another example with the propensity to have aberrant functioning of the *MYB* gene largely driven by chromosomal translocations. *MYB* rearrangement is pathogenic in AdCC being central to the slow progression of this ultimately fatal disease [5].

Accumulating evidence has shown that a patient's immune response is a major factor that can influence their response to disease progression most demonstrably in CRC and breast cancer [6–8]. In this context, recent evidence has clearly demonstrated that a specific reawakening of the latent immune response in several cancers can have a significant effect on improving tumour control. A key question is how this can be achieved for CRC and AdCC patients.

Over the past decade, pre-clinical research in our laboratory and a body of clinical observational studies have addressed this question. We have accumulated compelling data that suggests a DNA vaccination strategy may offer a potential immunotherapeutic option to treat tumours that display aberrant expression of the transcription factor MYB. This is achieved by harnessing the T-cell compartment of the immune system. It is this strategy and its translation to a clinical trial that is the subject of the current application.

1.2. TetMYB vaccine

Human *MYB* cDNA (containing three inactivating mutations in its reading frame) will be fused to *Tet* encoding cDNA. This fusion product is then cloned into the FDA compliant DNA vaccine vector pVAX1 to create the pVAX1-Tet-human MYB DNA vaccine (from here on referred to as TetMYB Vaccine). The TetMYB Vaccine was manufactured at the PMCC/VCCC in line with Good Manufacturing Practice (GMP) and FDA criteria for DNA vaccine production as specified by the FDA Centre for Biologics Evaluation and Research (CBER) document “Points to consider on Plasmid DNA vaccines for Preventative Infectious Diseases Indications” [9,10].

In addition, the facility and the manufacturing workflow for the production of the DNA vaccine has been subjected to a risk assessment analysis by Cell Therapies Pty Ltd, Peter MacCallum Cancer Centre, with recommendations to maximise the safety and integrity of the manufactured vaccine.

1.3. Anti-PD-1 antibody: BGB-A317

BGB-A317 is a humanised IgG4 variant monoclonal antibody against PD-1. It has been developed for the treatment of human malignancies. BGB-A317 was manufactured under Good Manufacture Practice (GMP) quality control systems. The clinical trial drug product is formulated in an aqueous buffer with pH 6.5 and isotonic osmolality. The suggested administration route is intravenous (IV) infusion after the appropriate dilution in 0.9% sodium chloride solution.

BGB-A317 binds to the extracellular domain of human PD-1 with high specificity and affinity (K_d = 0.15 nM) as demonstrated by receptor binding assays based on surface plasmon resonance. It competitively blocks the binding of both PD-L1 and PD-L2, inhibiting PD-1 mediated negative signalling in T-cells. In *in vitro* cell-based assays, the humanised antibody consistently and dose-dependently enhanced the functional activity of human T-cells and pre-activated, primary PBMCs (peripheral blood mononuclear cells). In addition, BGB-A317 demonstrated anti-tumour activity in several human cancer allogeneic xenograft models, including A431 human epidermoid carcinoma, BCCO-028 colon cancer, and BCLU-054 NSCLC models, where the PBMCs were co-injected with the human cancer cells (A431) or the tumour fragments (BCCO-028 and BCLU-054) into the immunocompromised mice.

The IgG4 variant antibody has very low binding affinity to FcγRIIIA and C1q by in vitro assays, suggesting a low or no antibody-dependent cellular cytotoxicity and complement-dependent cytotoxicity effect in humans. Unlike natural IgG4 antibody, BGB-A317 has no observable Fab-arm exchange activity by in vitro assay, predicting the antibody would be stable *in vivo*, and unlikely to form bispecific antibody.

2. Method/design

2.1. Objectives

2.1.1. Primary objective

- Stage 1 - Dose safety and escalation: To determine the safety, tolerability and the maximum tolerated dose (MTD) of the TetMYB Vaccine first as monotherapy, and then in combination with BGB-A317.
- Stage 2 - Expansion: To evaluate the efficacy of the combination of TetMYB Vaccine and BGB-A317 at the MTD.

2.1.2. Secondary objectives

- To evaluate $CD8^+$ and $CD4^+$ immunologic responses (as measured by an increase in MYB-specific T-cells) vs. evidence of clinical benefit immune response (as per irRECIST criteria, overall survival and progression-free survival).
- To assess the associations between functional imaging (FDG-PET) and CT/MRI (irRECIST) response.
- To assess the associations between the level of Tumour Infiltrating Lymphocytes (TILs) in biopsy samples with clinical outcomes (as per irRECIST criteria, overall survival and progression-free survival).

2.1.3. Exploratory objectives

- To explore mechanisms of tumour cell destruction through the use of matched T-cell population from TILs or PBMCs and tumour organoids grown from biopsy samples.
- To explore the potential utility of ctDNA and CEA to monitor disease response (irRECIST).
- To monitor immune cell subsets in peripheral blood, serum levels of cytokines, and antibodies against MYB.

2.2. Study setting and ethics review

This is a prospective single-arm, open-label, multi-centre Phase Ia/Ib study involving two institutions: Peter MacCallum Cancer Centre and Monash Health in Melbourne, Australia. The protocol has been reviewed and approved by the Peter MacCallum Cancer Centre Human Research Ethics Committee.

2.3. Endpoints

2.3.1. Primary endpoints

- Stage 1 - Dose safety and escalation: The incidence of Grade 3 or greater adverse events (AEs) and clinical laboratory abnormalities defined as dose-limiting toxicities (DLTs). Tolerability: defined as receiving a minimum of 4 doses of TetMYB Vaccine within 6 weeks of starting treatment.
- Stage 2 - Expansion: Objective immune response as defined by: achievement of a complete (irCR) or partial (irPR) response, based on irRECIST criteria within 12 weeks of first vaccination.

2.3.2. Secondary endpoints

- Safety profile for adverse events with a start date following 6 weeks of the first vaccination (occurrence, type, severity and relationship to treatment; described according to NCI CTCAE v4.03).
- Injection site reactions.
- Efficacy endpoints:
  - Objective response as defined by the achievement of a complete (irCR) or partial (irPR) response, based on irRECIST criteria within 12 weeks of first vaccination.
  - Clinical benefit as defined by:
    - Achievement of a complete (irCR) or partial (irPR) response as per irRECIST criteria; or
    - Maintenance of stable disease (irSD), as per irRECIST criteria, at 12 weeks after commencement of treatment.
  - Progression free survival (PFS): defined as the time from first vaccination to the earliest of date of disease progression as assessed by irRECIST or death from any cause.
  - Overall survival (OS): defined as the time from first vaccination to date of death from any cause.
- Demonstration of MYB-specific immune response ($CD8^+$ and $CD4^+$ immunologic response):
  - Through epitope stimulation of PBMCs and assessment with cytometric bead array and fluorescence-activated cell sorting for IFN-γ and TNF-α production, and
  - Evaluation of changes in tumour microenvironment (biomarkers: MYB expression & TILs) in pre- and post-treatment biopsies.
- Metabolic response, as assessed by FDG-PET scan.
- Immune cell subsets in peripheral blood at baseline, during treatment and follow up.

2.3.3. Exploratory endpoints

- To demonstrate in vitro T-cell homing and killing of tumour organoids; tumour biopsy will be taken at 4 weeks (day 1, week 5) from first vaccination for tumouroid production and archival storage for future research; this will be mandatory for CRC cohort but optional for AdCC cohort.
- To evaluate the role of ctDNA in efficacy assessment and disease monitoring; ctDNA (presence/absence and amount) will be evaluated together with CEA at baseline and monthly: T_{2x} (doubling-time) for progression and $T_{1/2}$ for regression.
- Serum levels of cytokines, antibodies to MYB at baseline, during treatment and follow up.

2.4. Study population

Patients with a diagnosis of advanced or metastatic colorectal or adenoid cystic carcinoma who meet all the inclusion and exclusion criteria will be eligible for participation in this study.

2.4.1. Inclusion criteria

1. Male or female aged 18 years or older at screening.
2. Patients with advanced/metastatic colorectal, adenoid cystic carcinoma, or other solid tumours known to express MYB; and for which no effective standard therapy is available.
3. Patients have been fully informed about the study and are willing to participate in the study, and have provided written informed consent prior to any trial specific screening procedures.
4. Measurable disease as per irRECIST Criteria 1.1.
5. Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 or 1.
6. Life expectancy greater than 3 months.
7. Adequate haematological, renal and hepatic functions as defined by:
   - Neutrophil count $>1.5 \times 10^9$/L
   - Platelets $>100 \times 10^9$/L
   - Hb >100 g/L (Patients can be transfused in the lead-in period, providing they do not have active bleeding or require regular transfusions).
   - Total bilirubin $<1.5 \times$ upper limit of normal (ULN)
   - ALT and AST $<2.5 \times$ ULN ($<5.0 \times$ ULN for patients with hepatic metastasis)
   - Serum creatinine $<1.5 \times$ ULN or Creatinine Clearance >50 mL/min (Cockroft-Gault or Nuclear GFR method)
8. Willing to provide study specific pre-treatment biopsy of tumour and allow use of archival tumour biopsies. Biopsies are mandatory unless discussed with the Study Chair.
9. Willing to consent to the use of their collected fresh tumour and archival FFPE specimen and blood samples as detailed in the protocol for research including but not limited to DNA, RNA and protein based biomarker detection.
10. Men and women of childbearing potential must use adequate contraception to prevent pregnancy during the study. Adequate contraception is defined in the study as any medically recommended method (or combination of methods) as per standard of care. An adequate contraception includes hormonal contraception with implants or combined oral, transdermal or injectable contraceptives, certain intrauterine devices, bilateral tubal ligation, hysterectomy, or vasectomy of partner. Combinations of male condom with either cap, diaphragm or sponge with spermicide are also considered acceptable. For women of childbearing age, a negative pregnancy test needs to be confirmed before inclusion.

2.4.2. Exclusion criteria

1. Prior therapy with an anti-cancer vaccine; or anti-PD-1, anti-PD-L1, anti-PD-L2, anti-CD137, anti-CTLA-4 antibody, or any other antibody or drug specifically targeting T-cell co-stimulation or immune checkpoint pathways.
2. Chemotherapy, radioactive, biological cancer therapy, or tyrosine kinase inhibitor (TKI) therapy, within four weeks prior to the first dose of study drug. All toxicities attributed to prior anti-cancer therapy other than alopecia and fatigue must have resolved to Grade 1 (NCI CTCAE v4.03) or baseline before administration of study drug.
3. Patients with a prior malignancy active within the previous 3 years except for locally curable cancers that have been apparently cured, such as basal or squamous cell skin cancer, superficial bladder cancer or carcinoma *in situ* of the cervix or breast, or localised

prostate cancer.
4. Patient has an autoimmune disorder or history of autoimmune disease requiring immunosuppressive treatment.
5. Uncontrolled or significant intercurrent or recent illness including:
   - Cardiac disorder such as uncontrolled cardiac failure, unstable angina or non-ST or ST segment elevation myocardial infarctions, or uncontrolled arrhythmia less than 3 months before screening.
   - Active or uncontrolled severe infection.
6. History of solid organ transplantation or any condition requiring chronic treatment with corticosteroids or other immunosuppressive agents.
7. Active coagulopathy/bleeding diathesis.
8. Cirrhosis, chronic active or untreated persistent hepatitis.
9. Active Hepatitis B: defined as having a positive Hepatitis B surface antigen [HBsAg] test at screening. Patients with past or resolved Hepatitis B infection (defined as having a negative HBsAg test and a positive IgG antibody to Hepatitis B core antigen [anti-HBc]) are eligible. Hepatitis B virus (HBV) DNA must be obtained in these patients prior to registration, and must demonstrate no active infection.
10. Active Hepatitis C: Patients positive for Hepatitis C virus (HCV) antibody are eligible only if polymerase chain reaction (PCR) is negative for HCV RNA.
11. History of adverse reactions to peptide vaccines.
12. Pregnancy or lactating.
13. Has received an investigational drug within 4 weeks prior to first dose of study drug, or unless other has been agreed with the Study Chair.
14. Is currently receiving any agent with a known effect on the immune system, unless at dose levels that is not immunosuppressive (e.g. prednisolone at 10 mg/day or less or as inhaled steroid at doses used for the treatment of asthma).
15. Known history of positive tests for HIV/AIDS.
16. Prior treated brain metastases must be without evidence of progression (through magnetic resonance imaging [MRI] with contrast - preferred method or contrast enhanced computed tomography [CT]) for at least 4 weeks and off immunosuppressive doses of systemic medications, such as steroids (doses > 10 mg/day prednisone or equivalent) for at least 2 weeks before study drug administration to be eligible.
17. Receipt of live, attenuated vaccine within 28 days prior to the first dose of study drug (Note: subjects, if enrolled, should not receive live vaccine during the study and 180 days after the last dose of study drug).
18. Any contraindication to receiving anti-PD-1 antibody (BGB-A317) or hypersensitivity to the constituents of BGB-A317.
19. Has a history or current evidence of any condition, therapy, or laboratory abnormality that might confound the results of the trial, interfere with the patient's participation for the full duration of the trial, or is not in the best interest of the patient to participate, in the opinion of the treating investigator.
20. Has known psychiatric or substance abuse disorders that would interfere with cooperation with the requirements of the trial.

2.5. Treatment methods

TetMYB Vaccine treatment will be given weekly for a total of 6 weeks unless it is ceased due to unacceptable toxicity, progressive disease or other factors. BGB-A317 treatment will commence on week 4 and continue every 3 weeks onwards while receiving clinical benefit or until unacceptable toxicity, progressive disease or other factors.

The study will involve two stages:

1) Stage 1: Dose Safety and Escalation

This stage includes patients with advanced/metastatic colorectal adenocarcinoma (CRC) and adenoid cystic carcinoma (AdCC) and aims to establish the Maximum Tolerated Dose (MTD). Patients will be treated in sequential dose escalation cohorts in a 1 + 2 design (modified 3 + 3) as follows:

a. TetMYB Vaccine monotherapy:

The first patient of each dose level will be treated with 6 doses of TetMYB Vaccine monotherapy over 6 weeks; after which there will be Safety Data Monitoring Committee (SDMC) evaluation before commencing treatment of further patients at that particular dose level. If there is a reported DLT (as per DLT rules) in the first patient, a further 2 patients will be evaluated with TetMYB Vaccine monotherapy and assessed as per DLT rules. If there is no DLT after 6 doses of TetMYB Vaccine monotherapy then the first patient will be commenced on 3 weekly BGB-A317.

b. TetMYB Vaccine + BGB-A317:

If there is no DLT reported in the first patient, patient 2 and 3 will be given 3 doses of TetMYB monotherapy followed by combination TetMYB Vaccine & BGB-A317 at the fourth dose, with TetMYB Vaccine continuing weekly for a total of 6 doses and BGB-A317 given every 3 weeks.

Within each escalation cohort, there will be an interval of at least 1 week between the first and second patient starting TetMYB Vaccine treatment. This is to increase the likelihood that an acute Adverse Event (AE) will be detected prior to dosing of the next patient.

There will be 3 dose levels:

Dose Level	Amount of DNA (mg)
1	0.1
2	0.5
3	1.0

The first dose to be evaluated will be dose level 1 and escalated to dose level 2 using a 1 + 2 design. Dose escalation/de-escalation decisions will be made by the SDMC.

Dose Limiting Toxicities (DLTs) will be monitored over the first six weeks of treatment in a series of 1 + 2 patient cohorts; 1 patient for TetMYB as monotherapy and 2 patients for combination TetMYB and BGB-A317. After each cohort completes the DLT observation period, a clinical synthesis of the available toxicity information (including DLTs, adverse events that are not DLTs, and adverse events post the DLT observation period), will be reviewed by the SDMC and will be used to determine the dose regimen for the next cohort. Recruitment will be temporarily halted after each cohort until the SDMC have met and a decision about dose escalation/de-escalation has been agreed upon.

DLT-evaluable patients will be defined as all eligible patients who either:

(i) received at least one dose of the TetMYB Vaccine in the dose safety and escalation stage at the dose level to which they were assigned and experienced a DLT during the 6-week DLT observation period; or
(ii) received a minimum of 4 doses of TetMYB Vaccine without experiencing a DLT

The first patient(s) of each dose level (receiving TetMYB monotherapy) will be evaluated alone, and then in the same cohort as those receiving combination therapy.

2) Stage 2: Expansion

This stage consists of two patient cohorts treated at the MTD:

a. A CRC Expansion Cohort of patients with advanced/metastatic CRC.
b. An Exploratory Expansion Cohort of patients for whom there is a rationale for treatment with a TetMYB Vaccine-BGB-A317 combination, with a focus on AdCC.

Once the maximum tolerated dose (MTD) is defined in the dose safety and escalation stage, recruitment will continue to expansion stage. Evaluable patients for the expansion stage will be defined as all registered, eligible patients who commence treatment with the TetMYB Vaccine at the MTD in combination with BGB-A317. Up to 6 patients with mCRC treated at the MTD in the dose safety and escalation stage will also comprise the first patients in the mCRC expansion cohort. Patients in both cohorts of expansion stage will remain on treatment until disease progression, unacceptable toxicity, patient discontinuation/withdrawal or at the discretion of the treating clinician, after which time they will be followed for survival only. The expected time to progression is 6 months and the expected additional survival time is up to 12 months; therefore, each patient is expected to be on study for approximately 18 months from registration.

Treatment beyond progression: Subjects with progressive disease (PD) that have been confirmed but is not worsening and with otherwise stable or improved clinical status may continue to be treated with study drug until there is further progression, defined as 10% or greater increase in tumour burden volume from time of initial progression (including all target lesions and new measurable lesions), or clinical deterioration. These patients should be discussed with the Study Chair within 1 cycle of continuing of treatment beyond progression.

2.6. Dose-limiting toxicity (DLT) and adverse events (AEs)

A DLT is defined as any treatment-emergent Grade 3 or higher adverse event whether considered related to study drug or not and that occurs during the DLT evaluation period. Adverse events that are clearly and directly related to the primary disease or to another aetiology are excluded from this definition. The DLT evaluation period will be from initial dose of study treatment to 6 weeks later.

The following events occurring during the DLT evaluation period will not be considered DLTs:

- Grade 3 infusion-related reaction (first occurrence and in the absence of steroid prophylaxis) that resolves within 6 hours with appropriate clinical management.
- Grade 3 inflammatory reaction attributed to a local anti-tumour response (e.g. inflammatory reaction at sites of metastatic disease, lymph nodes, etc.).
- Grade 3 fatigue lasting $\leq$7 days.
- Grade 3 endocrine disorder (thyroid, pituitary and/or adrenal insufficiency) that is managed with or without systemic corticosteroid therapy and/or hormone replacement therapy and the subject is asymptomatic.
- Concurrent vitiligo or alopecia of any AE grade.
- Grade 3 or 4 neutropenia that is not associated with fever or systemic infection that improves without medical intervention by at least one grade within 3 days. Grade 3 or 4 febrile neutropenia will be a DLT regardless of duration or reversibility.
- Grade 3 or 4 lymphopenia that is asymptomatic.
- Grade 3 thrombocytopenia that is not associated with clinically significant bleeding and does not require medical intervention, and improves by at least one grade within 3 days.
- Isolated Grade 3 or 4 elevations in amylase and/or lipase that are not associated with clinical signs or symptoms or radiographic features suggestive of pancreatitis.
- Isolated Grade 3 electrolyte abnormalities that are not associated with clinical signs or symptoms and are reversed to Grade 1 with appropriate maximal medical intervention within 3 days.

Immune-related Adverse Events (irAEs) are defined as AEs of an immune nature (i.e., inflammatory) in the absence of a clear alternative aetiology. In the absence of a clinically significant abnormality, repeat laboratory testing will be conducted to confirm significant laboratory findings prior to designation as a DLT. Based on the emerging safety profile, an AE not listed above may be defined as a DLT after consultation with the Study Chair.

A patient who experiences a DLT in the first 6 weeks of treatment, will permanently cease protocol treatment and enter into the follow-up phase of the study. There are no dose reductions or modifications permitted in the DLT observation period of the study.

2.7. Study assessments

Patients will be reviewed and assessed as per the Schedule of Events in Appendix 1.

2.8. Screening

Screening will be performed within 28 days prior to first dose of treatment unless otherwise specified. The following assessments will take place:

- Informed consent obtained
- Documentation of patient demographics, medical history, baseline abnormalities, prior anti-cancer-treatments received
- Full physical examination, vital signs, including height and weight, documentation of ECOG performance status
- Radiological evaluation with a CT/MRI Chest/Abdo/Pelvis and FDG-PET
- Laboratory studies (haematology & biochemistry) including: FBE, UECr, LFTs, TSH, LDH
- Viral serology including: HIV, HBV-DNA, HbsAg, HBsAb, HCV-RNA-PCR
- Tumour marker: CEA in CRC patients
- ECG for estimation of QTcF
- Collection of correlative samples (blood and tissue) for exploratory research

2.9. Treatment and end of treatment

The following assessments will be performed during treatment and at the end of treatment visit:

- Documentation of concomitant medications
- Full physical examination, documentation of ECOG performance status
- Vital signs, including weight
- Laboratory studies (haematology & biochemistry): FBE, UEC, LFTs, TSH, LDH
- Tumour marker: CEA in CRC patients
- ECG for estimation of QTcF
- Radiological evaluation with a CT (or MRI) Chest/Abdomen/Pelvis and FDG-PET
- Response assessment (according to irRECIST)
- Adverse events assessment as per CTCAE v4.03
- Collection of correlative samples (blood and tissue) for exploratory research

2.10. Follow-up

All patients will be followed up for survival and disease status 3 monthly ($\pm$7 days) until death or for a minimum of 18 months after the last patient has been registered. The following assessments will take place at each visit:

- Documentation of survival status
- Documentation of new anti-cancer therapies

2.11. Efficacy assessment method

Assessment of efficacy will be performed using the following criteria:

1) Clinical Assessment

All patients will remain on treatment until disease progression, unacceptable toxicity, patient discontinuation/withdrawal or at the discretion of the sponsor/investigator, after which time they will be followed for survival only.

Key parameters assessed will be ECOG status, vital signs, weight, and clinical examination.

2) Immunological/Histology Assessment

The measure of efficacy will be induction of cytotoxic T-cell mediated killing of transformed human cells that aberrantly express MYB protein. To determine such a response, we will initially evaluate two parameters. Firstly, we will isolate 40 mL of peripheral blood from patients prior to and then again before subsequent vaccine boosts.

PBMCs will be isolated from peripheral blood samples and used to interrogate a tiled array of overlapping MYB peptides that represent the T-cell reactive epitopes of the entire Tet-MYB protein expressed by the vaccine. This assay will not depend upon a need to match the patient's MHC subtype. The production of interferon gamma (IFN-γ), tumour necrosis factor alpha (TNF-α) and other cytokines will be assessed by intracellular and extracellular (cytokines bead array) analysis by flow cytometry, and ELISpot assay. This will indicate whether or not there has been a general and/or specific immune activation in response to the TetMYB Vaccine. Furthermore, we will collect supernatants from the co-culture of TILs/PBMCs and matched organoids (cytotoxic assay) to analyse cytokine secretion. Plasma will be stored at -80 °C for future ctDNA analysis.

Archival tumour tissue will be collected for purpose of histological examination and biomarker/immunology analysis. A fresh tumour biopsy at baseline, during the initial screening period, will be collected. A second biopsy will be taken at week 4 for comparative analysis.

3) Imaging

To assess associations of clinical and immunological efficacy of TetMYB Vaccine, as monotherapy or in combination with BGB-A317, with non-invasive tumour burden assessment, a CT (or MRI) scan will be performed at baseline, week 6, week 12, and every 9 weeks thereafter. Reporting will be based on the irRECIST criteria.

4) Overall Response using irRECIST

The Overall Response is derived from the responses in measurable lesions according to the irRECIST criteria. Given that pseudo-progression may occur due to immune cell infiltration and other mechanisms, progressive disease (PD) suspected by the Investigator as pseudo-progression may continue treatment until confirmation of PD with repeat imaging at least 4 weeks later or at the next regularly scheduled imaging timepoint, but not to exceed 12 weeks from the initial documentation of pseudo-progression PD.

Under these circumstances, the patient must be re-evaluated and the following criteria must be met in order to continue the treatment after initial PD:

a) Absence of clinical symptoms and signs of disease progression (including worsening laboratory values).
b) Stable ECOG performance status, relative to baseline.
c) Absence of rapid progression of disease or of progressive tumour at critical anatomical sites (e.g. cord compression) that necessitates urgent alternative medical intervention.

5) Metabolic Response (FDG-PET)

Metabolic response assessment will be performed using FDG-PET scans at baseline, week 4 and end of treatment; and optional at time of progression. PET scan will be recorded in site eCRF as completion status only. Raw scan data will be acquired from remote sites and sent to the Peter MacCallum Cancer Centre to be reported by a PET scan specialist. Metabolic response data is considered experimental data and will not be recorded in eCRF.

2.12. Statistical analysis

This is a Phase Ia/Ib study, where patients are all treated with the same treatment. The Dose Safety and Escalation Stage is based on a standard 3 + 3 design (1 + 2 design), aiming to assess safety and determine the MTD. The Expansion Stage consists of two patient cohorts (CRC and AdCC or other tumours), and aims to assess efficacy.

2.12.1. Analysis populations

Eligible Patients: all patients meeting eligibility criteria.

Dose Safety and Escalation Stage Evaluable Patients: patients registered into the dose safety and escalation stage of the study, who either:

a. Received at least one dose of the TetMYB Vaccine in the dose safety and escalation stage at the dose level to which they were assigned and experienced a DLT during the 6-week DLT observation period; or
b. Received a minimum of 4 doses of TetMYB Vaccine without experiencing a DLT.

The first patient(s) of each dose level (receiving TetMYB Vaccine monotherapy) will be evaluated alone, and then in the same cohort as those receiving combination therapy.

Expansion Stage Evaluable patients with CRC (CRC-expansion): all registered, eligible patients with CRC who commence treatment with the TetMYB Vaccine at the MTD defined in the dose safety and escalation stage and BGB-A317.

Expansion Stage Evaluable patients with AdCC or other selected tumours (Exploratory expansion): all registered, eligible patients with AdCC (or other tumours) who commence treatment with the TetMYB Vaccine at the MTD defined in the dose safety and escalation stage and BGB-A317.

DLT rates will be assessed in the expansion stage evaluable patients who were not recruited to the dose safety and escalation stage of the protocol (expansion cohorts).

2.12.2. Statistical methods

Baseline and treatment characteristics of the eligible patients in the trial who receive the vaccine will be summarised. All patients who come off study at any stage following registration (including those who do not receive the vaccination) will be reported. The reason of withdrawal will be documented and summarised.

The MTD and rates of DLTs and tolerability will be determined at the end of the dose safety and escalation stage of study. Rates of DLTs will be reported, with 95% confidence intervals, by dose level cohort in dose safety and escalation stage (based on dose received). There may be a bias here (as occurrence of a DLT had an influence on continuation or otherwise of recruitment to the dose level cohort), thus the rates of DLTs will also be reported among patients in the expansion cohorts.

For the main and final reports after dose escalation has been

completed, rates of tolerability and worst grade of adverse events, both reported and only those believed to be possibly, probably or definitely related to study treatment, will be calculated, with 95% confidence intervals, over all patients, among those treated at the MTD, and among those treated at less than the MTD.

The anti-tumour activity (efficacy) will be summarised across all patients (both dose safety and escalation, and expansion stages), and among those treated at the MTD, and among groups of patients with CRC and other diagnoses. Response rates (including objective response by irRECIST, clinical benefit by irRECIST and PET response) will be reported.

Amongst patients treated at the MTD, considering CRC and non-CRC patients separately, time to event (progression free survival and overall survival) analyses will be performed using Kaplan-Meier method for survival data, with a close out date applied. The close out date will generally be taken to be the earliest of the dates of last contact, at which the assessments relevant to the identification of the events were made, of the patients who are still alive and being followed up. Thus, with the exception of any patients who have been lost to follow-up, the status of all patients in the trial, regarding the events of interest, should be known at this date. The name of any anti-cancer therapy commenced after study treatment began will be tabulated.

The rates and 95% CI's of presence (and mean, standard deviation, median and range for quantity) of immune cell subsets in peripheral blood and serum levels of cytokines at each timepoint of assessment will be presented. The association between baseline functional imaging or biomarkers or tumour infiltrating lymphocytes and irRECIST response will be assessed by logistic regression; and with time to event outcomes assessed by Cox proportional hazards regression. Where measures taken at times other than baseline are to be assessed for association with irRECIST response or time to event outcomes, appropriate incorporation of time-varying covariates will be used. The significance level will be 0.05.

2.12.3. Sample size calculation and expected duration

In the dose safety and escalation stage of the study, the sample size is pragmatic in the modified 3 + 3 (1 + 2) design, and the adverse events will be estimated with the following levels of precision:

2.13. Precision around rates with 3 or 6 patients per cohort

Number of patients with adverse event in cohort	Rate with exact 95% confidence intervals if 6 patients in cohort	Rate with exact 95% confidence intervals if 3 patients in cohort
0	0% (0–46%)	0% (0–71%)
1	17% (0–64%)	33% (1–91%)
2	33% (4–78%)	67% (9–99%)

In the expansion stage of the study, it is assumed that a response rate of 0% is considered unacceptable and does not warrant further investigation of this treatment. It is desirable to have a response rate of at least 10% with the proposed treatment. A sample size of 15 patients is required to have 80% power with type I error rate of 0.05 to test the null hypothesis that the true response rate is 0% against an exact one-sided alternative of 10%.

If one patient experiences an objective response among 15 patients, the precision of the objective response estimate would be: 7% (95% CI: 0%–32%).

It is intended to recruit 5–17 patients in the exploratory expansion cohort (patients with selected solid tumours for which there is a strong rationale for treatment with this combination, with a focus on AdCC). With similar numbers of patients in this cohort, there will be similar precision to estimate the objective response among this cohort to the CRC-expansion cohort – this is an exploratory cohort with a pragmatic sample size.

2.14. Timing of analysis reports

Safety reports for the SDMC committee after the first patient and after every 3 patients in the dose escalation phase of the study. The final safety report will contain safety data about the MTD and rates of DLTs from the dose escalation phase of the study.

Main analysis report at 12 weeks after the final patient is recruited to the expansion cohort. This will contain baseline characteristics, treatment details, adverse events summaries, anti-tumour activity and correlative study data, if available.

Final analysis report once all the expansion phase patients treated at the maximum tolerated dose have experienced disease progression or withdrawn from study procedures for other reasons (e.g. end of study as 18 months since the last patient recruited). This will provide an update to the main analysis report where additional data has been reported.

3. Discussion

Activation of the MYB gene is seen in multiple malignancies: acute myelogenous leukemias, T-cell leukemias, colorectal carcinomas, estrogen-receptor positive breast cancer and pancreatic cancer; or gene fusion, *MYB-NFIB*, in the case of adenoid cystic carcinoma. By employing a DNA plasmid vector incorporating *MYB*, this novel technique overcomes the constraints of epitope/MHC restriction when targeting an endogenous antigen. The results from this study will be instructive in documenting the biology of a DNA vaccine and allow for development of assays to evaluate the immunologic and clinical efficacy of these vaccines in the future.

Furthermore, there has been a paucity of effective treatment for metastatic adenoid cystic carcinoma. If effective, TetMYB is a major breakthrough for these patients, who otherwise would suffer a prolonged and relenting disease course.

4. Trial status

Protocol Version 2.1: 31 January 2019. The MYPHISMO trial is currently recruiting.

Declaration

Ethics approval and consent to participate

This study and protocol have been approved by the Peter MacCallum Cancer Centre Human Ethics Committee (EC00235).

Consent for publication

Not applicable.

Availability of data and material

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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Foundation. BeiGene (BeiGene, Ltd.) will be supplying the BGB-A317 at no cost for the purpose of clinical trial.

Authors' contributions

TP, JD, RR, LP, LG have been involved in trial design and writing of the protocol.

RR, SR, LP, SS, PD have contributed to the design of experimental assays.

JD, BS, MM, AH are involved in recruitment of patients.

TA is involved in design of imaging analysis protocol.

EL has contributed to the statistical methods of the trial.

All authors have contributed to this manuscript and approved the final manuscript.

Abbreviations

eCRF	Electronic Case Report Forms
CEA	Carcinoembryonic antigen
CR	Complete Response
CTCAE	Common Terminology Criteria for Adverse Events
DLT	Dose Limiting Toxicities
ECG	Electrocardiogram
ECOG	Eastern Co-operative Oncology Group
eCRF	Electronic Case Report Form
eDC	Electronic data capture
FBE	Full blood examination
FDG-PET	Fluorodeoxyglucose – Positron Emission Tomography
FDG-PET-CT	FDG-PET computed tomography
GCP	Good Clinical Practice
GMP	Good manufacturing practice
Hb	Haemoglobin
HREC	Human Research Ethics Committee
irRECIST	Immune Related Response Evaluation Criteria in Solid Tumours
irCR	Immune Related Complete Response
irND	Immune Related No Disease
irNE	Immune Related Non-Evaluable
irPD	Immune Related Progressive Disease
irSD	Immune Related Stable Disease
IMP	Investigational medicinal product
MRI	Magnetic Resonance Imaging
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
OS	Overall survival
PBMC	Peripheral Blood Mononuclear Cells
PD	Progressive Disease
PET	Positron Emission Tomography
PI	Principal Investigator
PICF	Patient Information Sheet and Consent Form
PR	Partial Response
PS	Performance Status
QTcF	Fridericia's-corrected QT interval
RECIST	Response Evaluation Criteria in Solid Tumours
SD	Stable Disease
SDMC	Safety Data Monitoring Committee
TILs	Tumour Infiltrating Lymphocytes

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.conctc.2019.100409.

Appendix 1. Schedule of Events

Week	Screening Days (−28 to −1)	Treatment										End of Treatment Occurs 28 days post last dose of treatment (±7 days)	Follow Up 3 monthly for at least 18 months (survival only)
		1 (±3 days)	2 (±3 days)	3 (±3 days)	4 (±3 days)	5 (±3 days)	6 (±3 days)	7 (±3 days)	10 (±3 days)	13 (±3 days)	*Every 3 weeks* (±3 days)		
Written informed consent	x												
Demographics	x												
Documentation of Medical History & Baseline Abnormalities	x												
Prior Anti-Cancer Treatments	x												
Prior and Concomitant Medications	x	x	x	x	x	x	x	x	x	x	x		
Physical exam/vital signs[a]/ weight/ECOG/Height (height at screening only)	x	x	x	x	x	x	x	x	x	x	x	x	
Haematology[b]	x[b]	x	x	x	x	x	x	x	x	x	x	x	
Serum biochemistry[c]	x[c]	x	x	x	x	x	x	x	x	x	x	x	
Serology[d]	x												
Tumour Markers: CEA[e]	x	Week 13, and every 9 weeks thereafter										x	
Pregnancy Test	x												
ECG[f]	x	x		x			x	Week 13 and every 3 weeks thereafter				x	
Imaging CT (or MRI) chest/ abdo/pelvis[g]	x						x	Week 13, and every 9 weeks thereafter				x	
FDG-PET	x				x			At time of PD (optional)				x	
TetMYB Vaccine Administration		x	x	x	x	x	x						
BGB-A317 Administration					x[j]			x	x	x	x		
Adverse Events[h]		x	x	x	x	x	x	x	x	x	x	x	
Survival Status													x
Anti-Cancer Therapies													x
Identification of Archival Material Available	x												
Fresh Tumour Biopsy Collection[i]	x				x			At time of PD (optional)					
Peripheral Blood Collection[j]	x				x			Week 7, Week 13, every 9 weeks thereafter and at time of PD					

a. Vital signs: Heart rate, respiratory rate, temperature, blood pressure.
b. Haematology: FBE and diff. Laboratory tests need to be performed within 3 days of each treatment related visit, except for the first visit (week 1), when these can be performed within −7 days.
c. Biochemistry: UECr, Calcium, LFTs, TSH, LDH. Laboratory tests need to be performed within −3 days of each treatment related visit, except for the first visit (week 1), when these can be performed within −7 days.
d. Serology: HIV, HBV-DNA, HbsAg, HBs Ab, HCV-RNA-PCR within 28 days of 1st dose of TetMYB Vaccine.
e. Perform CEA tumour marker testing on CRC patients only.
f. Single ECG.
g. CT chest/abdomen/pelvis to occur at baseline, week 6, week 13, and every 9 weeks (3 cycles) thereafter until evidence of disease progression or until patient withdrawal for other reasons. *Note*: Both oral and IV contrast is required. In the event of known allergy to contrast, an MRI abdomen/pelvis may be performed as an alternative. Method of tumour assessment should be consistent throughout all visits. Additional scans as clinically indicated. CT scans need to be performed within 7 days of scheduled visits.
h. Documentation of adverse events including immunological adverse events.
i. Pre- and on-treatment biopsies will be performed at baseline (during screening) and at week 4 during treatment. These biopsies are mandatory unless discussed with the Study Chair. An optional biopsy will also be performed at the time of progression for those patients who are considered to have benefitted from study treatment - i.e. those patients with a PC/CR or prolonged Stable Disease beyond 12 weeks. Refer to the MYPHISMO Laboratory Manual for tissue sampling information.
j. Refer to the MYPHISMO Laboratory Manual for blood sampling information.
k. Excluding the first patient enrolled into each dose level. BGB-A317 commences at week 7 for the first patient enrolled into each dose level.

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