SingHealth

CLINICAL TRIAL PROTOCOL

PROTOCOL TITLE:
Phase 1/2 Trial integrating allogeneic NK cells in high-risk advanced Stage III–IV nasopharyngeal cancer patients

PROTOCOL NUMBER:
001

NCT NUMBER: NCT07247201
PROTOCOL VERSION: Version 6
PROTOCOL DATE: 16 January 2026

PRINCIPAL INVESTIGATOR:
Lim Chwee Ming

SITE PRINCIPAL INVESTIGATOR:
Lim Chwee Ming, Senior Consultant, SGH

1. BACKGROUND AND RATIONALE

Nasopharyngeal carcinoma (NPC) is an aggressive head and neck cancer, primarily endemic in Singapore, Southeast Asia, Southern China and North Africa. A large majority (~70%) of NPC patients present with locally advanced disease at diagnosis and carry a high risk of recurrence (~30%) following definitive chemoradiotherapy (CRT). These relapses are highly lethal, and current chemotherapies can prolong progressive-free survival by 6-8 months only. To reduce NPC-related mortality, preventive strategies aiming to reduce the risk of relapses with treatment intensification upfront should be more effective than delivering treatments after relapses have occurred.

Intensifying treatment in locally advanced NPC patients using adjuvant chemotherapy following conventional CRT is often not well-tolerated, given that these patients are still recuperating from the acute CRT toxicities. Conversely, immunotherapy options, such as cell-based therapy and immune checkpoint inhibitor, are good candidates of adjuvant treatments because they are very well-tolerated even when combined with chemotherapy.

In line with this notion, our preliminary results have shown that the addition of functional NK cells to standard chemotherapy improved the prognostic outcome from chemotherapy alone based on the decrease in tumour growth rate and the final tumour volume. Therefore, this clinical trial is to validate if addition of Natural Killer (NK) infusion to NPC standard of care can reduce relapses and metastasis. Since NPC is ubiquitously associated with the Epstein Barr Virus (EBV) infection, detectable post-treatment circulating EBV-DNA is well-documented to represent minimal residue disease (MRD), which portends a worse prognosis. Therefore, complete eradication of remaining circulating EBV-DNA NPC cells would eliminate MRD following treatment, thereby reducing the risk of future relapses.

With these rationale in mind, our aim is to perform a dose escalating clinical trial to identify the maximum tolerate dose of allogeneic “off the shelf” NK cells integrated into the current standard of care treatment of advanced NPC patients; and investigate if addition of allogeneic “off the shelf” NK cells in the concurrent and adjuvant setting can reduce the probability of having detectable plasma EBV-DNA after definitive treatment.

1.1 General Introduction

NK cell is a member of the innate lymphoid cells (ILC) that is able to recognize and eradicate tumour cells without engaging MHC class I [1, 2]. A critical component of our innate immune system, NK cells is our first line of defence against carcinogenesis by their constant surveillance and elimination of circulating tumour cells [3, 4]. For the past decade, NK cells are constantly being explored as a new cell therapy in cancer treatment [5, 6]. The success in using NK cells to eliminate leukaemia has render much promise and merits to extend its use to treat solid tumour. In the past five years, several Phase I studies were being reported demonstrating good tolerance to the dosage of 0.5 to 3 billion allogeneic NK cells (derived from in-vitro expansion from PBMC) in cancer patients with no significant GVHD symptoms [7-10]. In addition, a study two years ago documenting the success in eliminating brain metastasis from NPC after failed chemotherapy using periodic infusion of 2 billion of allogeneic NK cells [11].

1.2 Rationale and Justification for the Study

This is a phase 1 leading to a Phase 2 clinical trial investigating addition of allogeneic NK cells to conventional CRT for locally advanced NPC patients. We hypothesis that allogeneic NK cells are well-tolerated and safe when used in combination with conventional CRT in treating locally advanced NPC and lead to elimination of post-treatment circulating EBV-DNA from the current 25-30% to 10% among locally advanced NPC patients.

From a mechanistic perspective, during concurrent CRT, the NPC tumour will be killed, and fragments of the EBV positive NPC tumour cells will be released into the circulation. In usual instance, the host circulating NK cells will be responsible to “mop” up these circulating NPC cells that may be still viable. However, due to the effects of immune suppression from chemoradiotherapy, some of these circulating NPC cells may not be adequately eliminated, resulting in missed opportunity to completely eradicate these circulating NPC cells. These viable cells may enter into dormancy and only to re-emerge subsequently as clinically evident cancer relapses. Therefore, in our trial design, we have purposefully integrated the use of allogeneic NK cells during the concurrent (2 doses) and adjuvant (4 doses) phase.

We hypothesis that by supplementing NK cells in these critical phases, the circulating NPC cells can be effectively killed and eliminated. Additionally, we also have highly sensitive plasma EBV-DNA assays that will allow us to measure and track the circulating NPC cells during this trial. A summary of our mechanistic explanation of our study is presented in Figure 1.

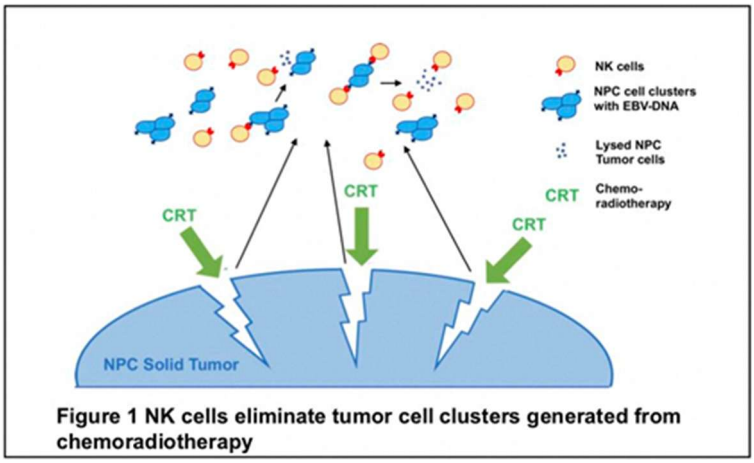


Figure 1: Mechanistic explanation to leverage on allogeneic NK cells to mop up circulating EBV-NPC cells following concurrent chemoradiotherapy. The lysed NPC cells following NK cell mediated cytotoxicity can further promote anti-NPC T cell responses from NK cell-dendritic cell cross presentation.

1.3 Rationale for the Study Purpose

A large majority (~70%) of Nasopharyngeal Carcinoma (NPC) patients present with locally advanced disease at diagnosis carry a high risk of recurrence (~30%) following definitive chemoradiotherapy (CRT). Preventive strategies with treatment intensification upfront should be more effective than delivering treatments after relapses have occurred. However, intensifying conventional CRT as adjuvant treatment in locally advanced NPC patients is often not well-tolerated, given that these patients are still recuperating from acute CRT toxicities. Conversely, NK cell therapy has shown to be very well-tolerated when use as sole therapeutic in cancer patients. Together with their capacity to eliminate tumour cells without engaging MHC class I or prior antigenic engagement, allogeneic NK cell is expected to be an ideal adjuvant immunotherapy to be combined with chemotherapy. To date however, such combinatory therapeutic approach towards cancer treatment has not been investigated for NPC.

In this clinical trial, we proposed a phase I study to determine the maximum Tolerated Dose (MTD) of allogeneic NK cells to be used in the trial, leading to a phase 2 study to evaluate the efficacy of such combined treatment approach on reducing cancer recurrence for NPC patients.

1.4 Rationale for Doses Selected

We anticipate the maximum tolerated dose (MTD) to be between $0.8x10^7$ to $1.8x10^7$ NK cells/kg (equivalent to approximately 0.5 to 1.0 billion NK cells) because this dosage range of allogeneic NK cells has been shown to be safe and feasible among several Phase I clinical trials [7-10]. Therefore, our team has adopted this in Phase I trial the same dosing regime in a carefully escalated manner.

1.5 Rationale for Study Population

We are selecting only locally advanced NPC patients who are at high risk of developing relapses in the future. Based on previous studies across many tertiary centres, locally advanced NPC patients (i.e. Stage III-IVA/B, T1-4, N1-3, M0) are at higher risk of future cancer relapse following current standard of care treatment with concurrent CRT. With a larger tumour burden, we believe that addition of allogeneic NK cells can help to “mop” up the circulating EBV NPC cells during and after the standard of care CRT treatment.

1.6 Rationale for Study Design

In Phase 1 study, we will determine the MTD using the Bayesian Model Averaging continual reassessment method (BMA-CRM) study design [12, 13] where **5 doses ($0.5x10^7$, $0.8x10^7$, $1.1x10^7$, $1.4x10^7$ and $1.8x\ 10^7$cells/Kg)** of NK cells will be evaluated. The primary goal of the BMA-CRM is to identify the maximum tolerated dose (MTD) of NK cells to be infused. An anticipated size of 6 patients will be accrued for the Phase 1 study. If the MTD is achieved earlier with smaller cohort of patients, we will proceed with phase 2 portion without having to complete the projected 21 patient's accrual on the phase 1 design.

The Phase 2 study is a single-arm trial derived from the anticipated proportion of locally advanced NPC patients (25-30%) who will continue to present with detectable circulating EBV-DNA following standard of care CRT paradigm. We hypothesize that in this single arm trial, adding allogeneic NK cells will completely eliminate the proportion of patients with detectable circulating EBV-DNA from 25-30% to 10%.

Hence, applying the one proportion Z-score test, a sample size of 9 will achieve 80.5% power to detect a difference (P1-P0) of -0.2500% using a one-sided exact test with a significance level (alpha) of 0.05. Assume the withdrawal rate at 0.2 (20%), the total sample size is estimated at 12. In our phase 1 design, at least 6 patients will be treated at our MTD of allogenic NK cells, meaning that additional 25 patients will be accrued in the phase 2 trial.

2. HYPOTHESIS AND OBJECTIVES

2.1 Hypothesis

The hypothesis of this trial is allogeneic NK cells will be well-tolerated and safe when used in combination with conventional CRT in locally advanced NPC patients leading to the elimination of post-treatment circulating EBV-DNA from the current 30% to 10% in locally advanced NPC patients.

2.2 Primary Objectives

The primary objective of this trial is to determine the MTD of allogeneic NK cells that is deemed safe and tolerable when used with concurrent CRT in advanced NPC patients

2.3 Secondary Objectives

The secondary objective of this trial is to determine if adding allogeneic NK cells to NPC standard of care CRT treatment paradigm can decrease the percentage of NPC patients' conversion to undetectable plasma EBV-DNA levels from 30 to 0%. The time to relapse will also be measured as an additional secondary endpoint.

2.4 Potential Risks and Benefits

2.4.1 Potential Risks

Allogeneic NK cells are generally safe to administer with minimal toxicities reported. The risk of graft versus host disease (GVHD) are generally very low, in comparison with T cell therapy. As part of the release criteria of our NK cells, we will ensure that the **minimum purity of NK cells to be at least 90%,** before these cells are administered to patients. Once our MTD of NK cells is ascertained, this dose will be delivered in the phase 2 portion of the study.

2.4.2 Potential Benefits

Integrating NK cells into CRT can potentially reduce the risk of future cancer relapse of NPC patients receiving definitive treatment. This risk can be objectively measured using circulating plasma EBV-DNA post treatment. Therefore, in this trial, we will measure the

circulating plasma EBV-DNA longitudinally and ascertain if the addition of NK cells can reduce the risk of having detectable plasma EBV-DNA following current standard of care treatment of CRT.

3 STUDY POPULATION

3.1 List the Number and Nature of Subjects to be Enrolled

We intend to accrue total of 31 locally advanced NPC patients for this Phase I leading to Phase II study. These patients are newly diagnosed advanced non-metastatic NPC patients who are at high risk of future cancer relapse.

In addition, during the early Phase I portion, we aim to recruit 40 to 70 healthy blood donors from general public and relatives of NPC patients. The collected blood from these individuals will serve as starting source materials (PBMCs) for NK cell manufacturing. All donors must meet the study's eligibility criteria and complete the required pre-screening assessments prior to participation.

3.2 Criteria for Recruitment and Recruitment Process

We plan to enrol up to 31 patients (6 in Phase 1 and 25 in Phase 2) over a period of 24–36 months from the following institutions: Singapore General Hospital, Sengkang General Hospital, Changi General Hospital, and the National Cancer Centre Singapore (NCCS). Newly diagnosed NPC patients with Stage III-IVA/B (T1-4, N1-3, M0)) disease and planned to start standard of care treatment will be identified and recruited. The detailed inclusion and exclusion criteria are outlined in Sections 3.3 and 3.4, respectively. Patients meeting the eligibility criteria will be approached to provide informed consent for participation in this clinical trial.

In parallel, we plan to enrol approximately 40 to 70 healthy volunteers from the general public and relatives of NPC patients. Potential donors will undergo pre-donation screening within seven days prior to the scheduled blood collection date. Individuals with a history of blood transfusion, major surgery, or any contagious disease within the past six months will be excluded. Donors must also abstain from taking any medications for at least 48 hours prior to blood collection. Only volunteers who meet all inclusion criteria and successfully pass the screening procedures will be invited to donate blood for the study.

3.3 Inclusion Criteria

a) Inclusion Criteria for study patient (NPC patient) as follows:
   - Age 21-80
   - NPC Stage III-IVA/B (T1-4, N1-3, M0)
   - Able to tolerate conventional CRT
   - Detectable EBV level at diagnosis
   - Adequate organ function
     - ANC ≥ 1500/μL
     - Platelet count ≥ 100,000/μL
     - Creatinine clearance ≥60ml/minute
     - Total bilirubin ≤ 1.5 x upper limit normal (ULN)
     - AST ≤ 2 x upper limit normal

  - ALT ≤ 2 x upper limit normal
- ECOG performance status of 0-1

b) Inclusion Criteria for Healthy Volunteers as follows:
- Age 21-50
- Body Mass Index (BMI): 19.0–30.0 kg/m²
- Haemoglobin level
  - Female: > 12.5 g/dL
  - Male: > 13g/dL.

3.4 Exclusion Criteria

a) Exclusion Criteria for study patient (NPC patient) as follows:
- Presence of Autoimmune Disease
- Any conditions resulting in Immunocompromised state (Drug induced)
- ECOG performance status ≥ 2
- Poor organ function
- Lactating or pregnant
- Serious concomitant disorders that would compromise the safety of the patient or compromise the patient's ability to complete the study.

b) Exclusion Criteria for Healthy Volunteers as follows:
- Lactating or pregnant
- History of malignancy
- History of blood transfusion or major surgery within the past 6 months
- History of any contagious or transmissible disease within the past 6 months
- Close contact with individuals diagnosed with any transmissible or contagious disease within the past 6 months

3.5 Subject Replacement

An accrued patient who fails to receive the first NK cell infusion during treatment will be considered non-evaluable and will be replaced.

Similarly, a healthy volunteer whose screening results are positive for any listed contagious or transmissible disease, or who fails to meet the eligibility criteria, will not proceed to blood donation and will be replaced.

4 STUDY DESIGN

This Clinical Trial comprised of an open label Phase 1 study leading to a single arm Phase 2 study.

Healthy volunteers will be recruited either through IRB-approved brochures or, in cases where the volunteer is a relative of a study participant, the PI/Co-I will communicate with them directly to explain the study details. All healthy volunteers will undergo screening for contagious or transmissible diseases prior to blood donation. Approximately 300–400 mL

of whole blood will be collected per donation using EDTA-containing infusion bags, from volunteers who have been screened and confirmed eligible for blood donation.

All collected blood units will be immediately transported to the designated GMP facility (ACTRIS) for processing and isolation of peripheral blood mononuclear cells (PBMCs). The isolated PBMCs will be aliquoted and subjected to quality testing, followed by cryopreservation using CS10 media. The cryopreserved PBMCs will be stored in liquid nitrogen at the ACTRIS facility. Once a sufficient PBMC cell yield is obtained, the cells will be processed for NK cell manufacturing. The final expanded NK cell product will be cryopreserved in liquid nitrogen and will only be reformulated on the day of infusion, after which it will undergo final quality control (QC) testing. The final formulated allogeneic NK cell product will then be administered to the NPC patient only.

For the Phase 1 study:

Determination of the maximum tolerated dose (MTD) using Continual Reassessment Method (CRM).

1. Doses to be evaluated (0**.5x10^7, 0.8x10^7, 1.1x10^7, 1.4x10^7 and 1.8x 10^{7}cells/kg**).
2. The starting dose to be evaluated is **0.8x10^7 cells/kg.**
3. Final allogeneic NK cells would be reformulated in a single infusion bag containing Plasma-Lyte 148, Interlukin-2 (200 IU/ml), and 2% Human Serum Albumin (HSA).
4. Dose Limiting Toxicity (DLT): Grade 3 to 5 toxicities (using Common Terminology Criteria for Adverse Events CTCAE).
5. Target Toxicity Limit (TTL): maximum probability of DLT occurring before rejecting the dose is set at 30% (0.3).

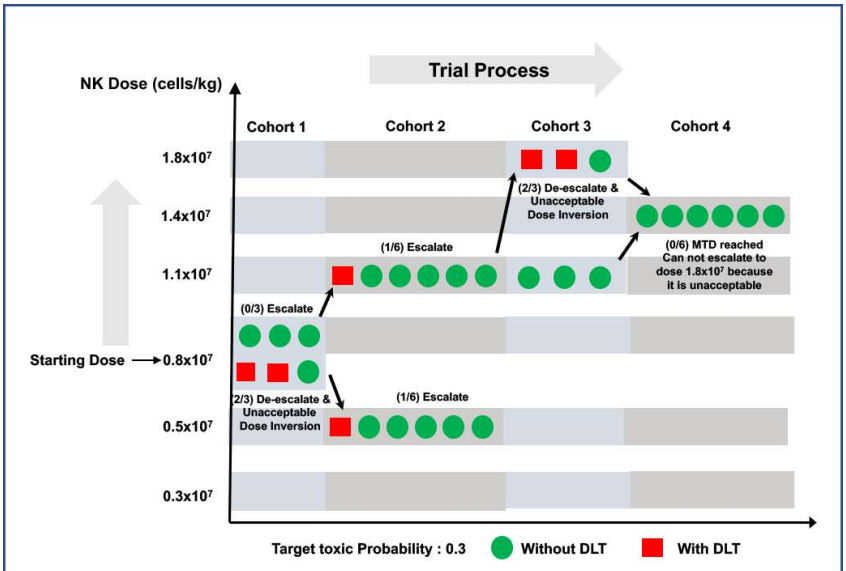


Figure 2 BMA-CRM methodology of achieving MTD of NK cells

For every case of DLT encountered, the toxicity limit will be reassessed to ascertain whether it has reached the TTL. In the instance when the cohort size is reached and the toxicity limit ≤ TTL, the dose will be escalated for MTD evaluation. Otherwise, if the toxicity limit > TTL, current dose will be rejected as MTD and the dose will be de-escalated for MTD evaluation. When the toxicity limit is very low, the dose escalation for MTD evaluated can be accelerated. i.e. escalating from dose 2 to 4 as shown in Figure 2. When 6 patients

treated at our set MTD do not develop grade 3-5 toxicities, the phase 1 portion of our study will be deemed to be completed, and then the set MTD will be accepted. **Every accrued patient involved in MTD evaluation will undergo the standard NPC treatment with the NK cells infusion dose set at the previously evaluated dose.**

For the Phase 2 study:

1. Standard Chemoradiotherapy will be implemented to every accrued NPC patient.
2. The dose of NK cells used will be set at MTD or the dose where the patient is subjected during Phase 1.
3. NK cells infusion will be carried out 1 time/week at **week 3, 6, 7, 8, 9 and 10.**
4. Final allogeneic NK cells would be reformulated in a single infusion bag containing Plasma-Lyte 148, Interlukin-2 (200 IU/ml), and 2% Human Serum Albumin (HSA).
5. Primary endpoint is set at the percentage of patients with detectable post-treatment circulating EBV-DNA has decreased from 30% to 10%.
6. Secondary endpoint is in determining the change in patient survival and recurrence rate within 24-36 months post-treatment under this new treatment regimen when compare with historic cohorts under standard treatments.

The

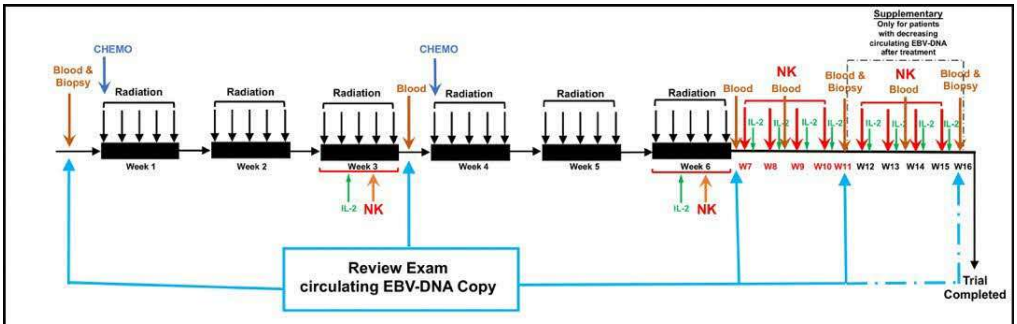


Figure 3 Treatment Regimen of the Clinical Trial

treatment regimen for the single arm Phase 2 study is showed in Figure 3. The 6 doses of NK cells will be infused once a week at specific week of treatment into every accrued patient. IL-2 will be co-formulated with the allogeneic NK cells with Plasma-Lyte148 and 2% HSA as a single ready for infusion product. At Week 9, patients who's circulating EBV-DNA levels have decreased but remain detectable will have the option to receive an additional four doses of NK cell infusions, administered from Week 12 to Week 15.

4.1 Randomisation and Blinding

This is as Phase 1 leading to single arm Phase 2 clinical trial. Hence, there will not be any randomization or blinding involved.

4.2 Contraception and Pregnancy Testing

For this trial, pregnant woman is a criterion for exclusion. Pregnant patients are excluded from receiving chemoradiotherapy as the standard of care CRT treatment. All females of reproductive age will be undergoing a pregnancy test as standard clinical care prior to receive CRT.

4.3 Study Visits and Procedures

(a) Study Visits and Procedures for Healthy Volunteers as follows:

Healthy Volunteers who indicate interest in participating in the study will be approached and the information about the study would be provided. Potential donors will be invited for in person visit, during which written consent would be obtained before carrying out any study related activities. The potential donors would be requested to complete a questionnaire which covers on medical history and lifestyle risk assessment. Then 20ml of blood would be collected for screening test to test for infectious disease. The screening result will be reviewed by PI/study team and are valid for 7 days. Donors who meet the eligibility criteria and have been tested negative will return within the 7-window period for the blood donation visit.

During the blood donation visit, eligibility will be reconfirmed, and vital signs and basic physical examination would be carried out prior to donation. Once 300-400 ml of blood is collected, the donors will be observed for 10-15 minutes to ensure that they are stable and safe to dismissed.

(b) Study Visits and Procedures for Study patient (NPC patient) as follows:

A table of scheduled study assessments for screening is provided in Table 1, Table 2 and Appendix 1. The Study will conclude ~ 4 months after accrual. Patients following treatment will be followed up as per normal standard of care by their treatment physician at both NCC and their respective treating ENT specialists. All assessments must be performed and documented in the medical record for each patient.

Screening evaluations will be performed ≤ 28 days before enrolment. Patients who agree to participate will sign the ICF before undergoing any screening procedure. The screening period begins on the date the ICF is signed. Screening evaluations may be repeated as needed within the screening period; the investigator will assess patient eligibility according to the latest screening assessment results. Results of standard-of-care tests or examinations performed before obtaining informed consent and ≤ 28 days before enrolment may be used for the purposes of screening rather than repeating the standard-of-care tests unless otherwise indicated. Voluntary, written informed consent for participation in the study must be obtained before any study-specific procedures are performed.

ICFs for enrolled patients and for patients who are screened but not enrolled will be maintained at the study site. All screening evaluations must be completed and reviewed to confirm that patients meet all eligibility criteria before enrolment.

The investigator will maintain a screening log to record details of all patients screened and to confirm eligibility or record reasons for screening failure, as applicable.

During the Screening Visit, a complete physical examination will be conducted, including evaluations of 1) head, eyes, ears, nose, and throat, 2) cardiovascular, 3) dermatological,4) musculoskeletal, 5) respiratory, 6) gastrointestinal, and 7) neurological

systems. Any abnormality identified during screening will be graded according to NCI-CTCAE v5.0 and recorded.

At subsequent visits (and as clinically indicated), limited, symptom-directed physical examinations will be performed. New or worsened clinically significant abnormalities are to be recorded as AEs.

The tissue biomarker sample collection schedule is described in Appendix 1 and in the Schedule of Assessments in each subprotocol (after screening). Archival tumour tissues should consist of FFPE blocks or approximately 15 freshly cut unstained slides. If no archival tumour tissues are available, a fresh tumour biopsy at baseline (during screening period) is mandatory. Blood samples will be taken predose on Day 1 to measure the circulating plasma EBV-DNA and other correlative markers as detailed.

Table 1

Assessment	**Screening [a]**	**Notes**
Days	**-28 to ~ -1**	
Informed consent	x	
Inclusion/exclusion criteria	x	
Enrolment	x	
Demographics/medical history/prior medications	x	Includes age or year of birth, gender, self-reported race/ethnicity, tobacco consumption (ie, former or current or never) and history of treatment for the primary diagnosis, including prior medication, locoregional treatment plan. Information on radiographic studies performed before study entry may be collected for review by the investigator. Preexisting AEs at baseline should be recorded as medical history.
Vital signs/ height and weight	x	Vital signs include body temperature, pulse rate, and blood pressure (systolic and diastolic). Pulse rate and blood pressure will be collected.
Physical examination	x	A complete physical examination is required.
ECOG Performance Status	x	
Adverse events	x	After the informed consent form has been signed but before the first administration of any study drug, only SAEs should be recorded.
Concomitant medications	x	
Haematology	x[a]	

Serum chemistry	x[a]	Local laboratory assessments on haematology, serum chemistry, coagulation parameters, and urinalysis will be conducted, of which certain elements will be collected.
Assessment	**Screening [a]**	**Notes**
Days	**-28 to ~ -1**	
Fresh tumour tissue	x	Pre-treatment Tissue will be collected either at time of diagnosis or after diagnosis, prior to to starting treatment.

4.4 Screening Visits and Procedures

Patient accrual will be in accordance with the following chart showed in Figure 3. Only NPC patients who have not previously received CRT treatment for NPC will be eligible for the clinical trial. In addition, they must meet all inclusion criteria and do not fall within the exclusion criteria will be invited to give consent to participate in this clinical trial. After consent is given, the NPC patients will be accrued to start the trial.

Moreover, healthy volunteers will be screened within seven days prior to the schedule donation date. Only donors who have tested and confirmed negative for infectious disease list below within seven (7) days of donation would be allowed to donate blood. Individuals who have undergone blood transfusion or major surgery or who have had any contagious disease in the past 6 months will be excluded. Donors must also refrain from taking any medications for at least 48 hours prior to blood collection.

20ml of potential donors' blood would be collected and would be tested for HIV-1/2 antigen and antibody, Human T-lymphotropic virus types I/II (HTLV-I/II), Syphilis, Cytomegalovirus (CMV) IgM and IgG, Hepatitis B virus (HBV), Hepatitis C virus (HCV), and Hepatitis E virus (HEV) and Vesicular stomatitis virus (VSV) IgG.

4.5 Study Visits and Procedures

1. All patients undergoing the trial will undergo conventional CRT as the standard treatment for NPC.
2. For patients who are accrued for the Phase 1 study, the dose of NK cells used in the treatment will be the same as the dose be evaluated in the determination of MTD. Once MTD is established, it will be used as the dose for all patients accrued subsequently.
3. Under the standard of care NPC treatment, chemotherapy will be carried out on week 1 and week 4. In addition, radiation treatment will be implemented daily Monday to Friday (2 Gyc per day) on week 1, 2, 3, 4, 5 and 6.
4. NK cells will be infused intravenously on week 3, 6, 7, 8, 9 and 10.
5. Interlukin-2 (200U/ml) will be delivered together with the NK cells in saline during NK infusion.
6. A tumour tissue biopsy will be taken at screening period (<28 days before starting treatment) and at 11.
7. Blood (20 ml) will be taken at screening period (<28 days before starting treatment) before treatment starts (week 0) and at week 4, 7, 9 and 11.
8. For patients who are undergoing supplementary NK treatment at the end of the trial, four additional doses of NK cells will be infused by their early dosage respectively once a week on week 12, 13, 14 and 15.
9. For patients receiving supplementary NK cells treatment, an additional blood (20 ml) and tumour tissue biopsy will be taken on week 16.

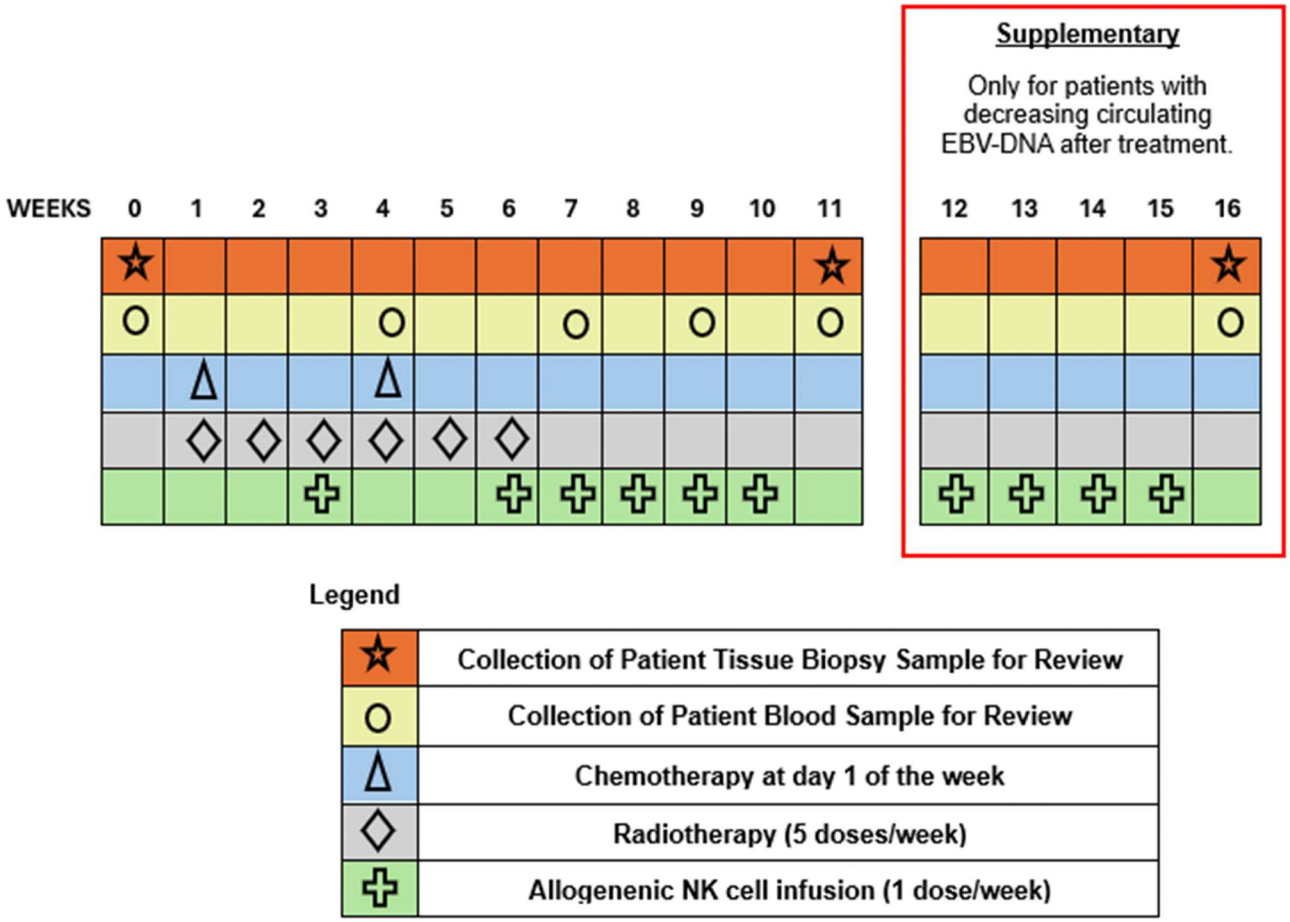


Table 2

Assessment Period	Assessment Procedures	Notes
-28 to -1	1. Draw Blood (20ml) 2. Tumour Tissue Biopsy 3. EBV assay	
Week 1	1. Chemo-drug Administration 2. Radiation Therapy Administration (1 dose daily, 5 doses/week)	
Week 2	1. Radiation Therapy Administration (1 dose daily, 5 doses/week)	
Week 3	1. Radiation Therapy Administration (1 dose daily, 5 doses/week) **2. NK cells infusion**	
Week 4	1. Draw Blood (20ml) * 2. EBV assay 3. Chemo-drug Administration 4. Radiation Therapy Administration (1 dose daily, 5 doses/week)	*Draw blood before Chemo-drug administration but they may not be on the same day.
Week 5	1. Radiation Therapy Administration	

	(1 dose daily, 5 doses/week)	
Week 6	1. Radiation Therapy Administration (1 dose daily, 5 doses/week) **2. NK cells infusion**	
Week 7	1. Draw Blood (20ml) * 2. EBV assay **3. NK cells infusion**	*Draw blood before NK cells infusion but they may not be on the same day.
Week 8	**1. NK cells infusion**	
Week 9	1. Draw Blood (20ml) * 2. EBV assay **3. NK cells infusion**	*Draw blood before NK cells infusion but they may not be on the same day.
Week 10	**1. NK cells infusion**	
Week 11	1. Draw Blood (20ml) 2. Tumour Tissue Biopsy 3. EBV assay	
Week 12 (Optional)	**1. NK cells infusion**	
Week 13 (Optional)	**1. NK cells infusion**	
Week 14 (Optional)	**1. NK cells infusion**	
Week 15 (Optional)	**1. NK cells infusion**	
Week 16 (Optional)	1. Draw Blood (20ml) 2. Tumour Tissue Biopsy 3. EBV assay	

4.6 Final Study Visit

At the end of the trial, the patients will be reviewed for their tolerability and safety of the allogeneic NK cell infusion.

4.7 Post Study Follow up and Procedures

As part of standard of care NPC surveillance protocol, both clinical and radiological assessments will be performed as follow:

a) **Clinical Assessments:** Physical examination (including flexible nasopharyngoscopy to examine the nasopharynx) will be performed monthly for the first year and bi-monthly for the second and third year as part of the routine NPC surveillance protocol.

b) **Radiological Assessments:** Baseline radiological imaging for tumour assessment and staging will be performed. Post-treatment radiological imaging will also be performed at 4-6 months post completion of treatment. Subsequent radiological studies will be performed as clinically indicated, determined by the treating physicians. The Response Evaluation Criteria in Solid Tumours (RECIST 1.1 criteria) will be used for assessment of response to therapy (from baseline to 4–6-month post treatment scans).

4.8 Discontinuation/Withdrawal

4.8.1 Discontinuation Criteria

Withdrawal Criteria:

1. Documented disease progression
2. Death
3. Development of an unacceptable toxicity, which in the opinion of the PIs, would render continued participation harmful.
4. Research subject or LAR withdraws consent from the study.
5. Investigator withdraws the subject from the study, in subject's best interest.
6. Non-compliance with protocol medications/administrations and/or required follow-up as judged by the PI.
7. Unable to be contacted and/or effectively monitored by the PIs and/or designees for follow-up (lost to follow-up).
8. Unable to receive the study infusion due to development of a significant health disorder, which in the judgment of the PI, would make proceeding with this study intervention to be not in the best medical interest of the participant.
9. Receipt of any therapy specifically for treatment of primary disease not defined in this protocol.

4.8.2 Discontinuation Visit and Procedures

Subjects may also withdraw voluntarily from receiving the study intervention for any reason during the study and they will continue to receive standard of care treatment for their cancer and follow up. Patients who withdraw from the study can give consent for their archival tissue samples to be used for correlative analyses with those who have completed the trial.

5 TRIAL MATERIALS

5.1 Trial Product(s) and Overview of NK Cell Manufacturing

The source of the allogeneic NK cells includes:

1. Healthy Donor from the public
2. Donor from Patients healthy relatives
3. GMP graded leukopaks.

A) Collection and Handling Donated Blood

Approximately 300–400 mL of whole blood will be collected per donation using EDTA-containing infusion bags. All collected units will be transported immediately to the designated GMP facility for isolation of peripheral blood mononuclear cells (PBMCs). The isolated PBMCs will be aliquoted, and quality tested before freezing in CS10 media and store in Liquid Nitrogen.

B) Manufacturing of Allogeneic NK cells

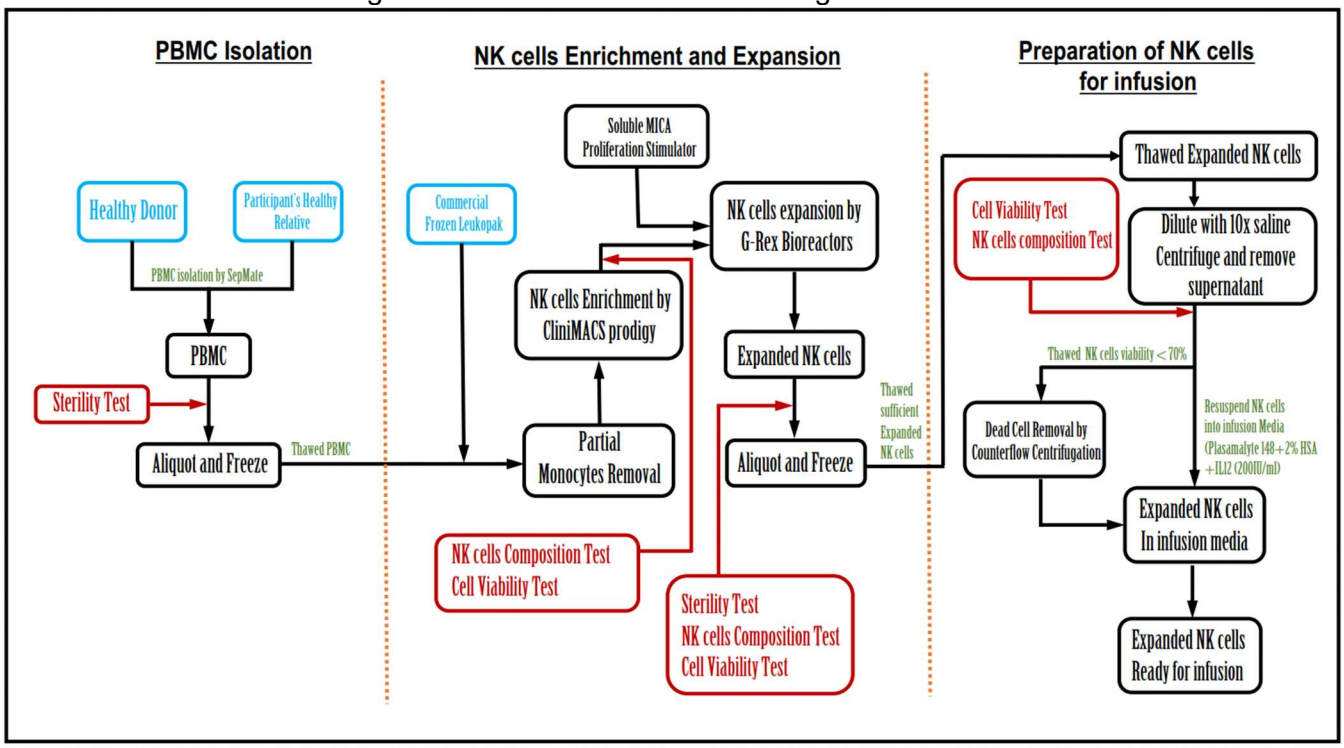
Manufacturing of the Allogenic NK cells will be performed by ACTRIS- our national cell manufacturing facility.

This process includes:

- Isolation of PBMC from whole blood
- Enrichment and Expansion of NK cells from the isolated PBMCs
- Preparation and formulation of allogenic NK cells

C) Overview of NK cell manufacturing

The NK cell Manufacturing workflow is shown in the flow diagram below.



1. Blood is obtained from:
   A) consented healthy relative of cancer patients (300 ml - 400ml)
   B) consented healthy public donor (300-400ml)
   C) Commercial GMP leukopaks.
2. PBMC will be isolated from whole blood via SepMate 50.
3. The isolated PBMC will be analysed for Sterility test (QC1).
4. PBMC will be aliquoted and frozen at $5x10^6$ cells/cryovial.
5. For batches of PBMC that did not clear the sterility test will be disposed in compliance with the Institutional guidance.
6. When sufficient PBMC cells are accumulated, they will be thawed and undergo NK cells enrichment using the automated CliniMACS Prodigy system.
7. The NK cells enrichment is achieved using CliniMACS CD3/CD56 complete kit used in conjunction with the automated CliniMACS Prodigy system.
8. After enrichment, a sample of the purified NK cells will undergo the Viability and Composition test (QC2).
9. The purified NK cells will be immediately used for in-vitro NK cells expansion.
10. Expansion of NK cells will be carried out in G-Rex100M open system Bioreactors.
11. Proliferation of NK cells is stimulated by cytokine cocktail (IL-15(50ng/ml) and IL-18(250ng/ml)) + MICA (50ng/ml)
12. Cell count will be determined on a regular basis (every 3-4 days).

13. When the cell density is ≥ 1x10^6 cells/ml NK cells culture will be distributed to 5 G-Rex100M Bioreactors to enable further expansion.
14. Three days later, 300ml of NK MACS media suppled with 5% Human AB serum and IL-2 (200IU/ml) are added to each G-Rex100M bioreactor.
15. Two days later, 500ml of NK MACS media suppled with 5% Human AB serum and IL-2 (200IU/ml) are added to each G-Rex100M bioreactor to a volume of 1000ml.
16. Cell count is performed every 3-4 days.
17. After another 10-14 days when the cell density is **≥** 2.5 x10^6 cells/ml, expanded NK cells will be harvested for QC testing.
18. Spent medium (40mL) will be sent for sterility test.
19. Also, samples of cells will be tested for Purity and Viability (QC3) as well as a cell count.
20. Then, expanded NK cells will be aliquoted and freeze at 5x10^8 cells/ml in 20 ml of CS10 per freezing bag.
21. On the day of NK cells infusion, sufficient bags of cryo-preserved expanded NK cells will be thawed and dilute 10 volumes of 0.9% saline supplemented with 5% Human serum albumin (HSA).
22. Centrifuge the thawed NK cells (500xg, 10 mins, 4°C) to pelletize the cells. Then, the supernatant is removed.
23. A small sample of the thawed NK cells will undergo test for purity and Viability (QC4).
24. Resuspend the rest of the NK cells into infusion media (Plasmalyte 148, HSA (2%), IL-2 (200IU/ml)).
25. Store the cells at 4°C (no more than 6 hours).
26. If the viability of the NK cells <70%, NK cells viability can be increased via counterflow centrifugation using the Rotea system.
27. Any excess expanded NK cells will be passed to the research lab.

5.2 Manufacturing Methodologies

A) Isolation of PBMC from blood followed by NK cells enrichment:

Materials:

- The source of Blood will be from consented healthy relative of cancer patients (300 - 400 ml);
- Consented healthy public donor (300-400ml) or Commercial GMP leukopaks.
- SepMate 50 (StemCell Technologies, cat no: 86450).
- Ficoll-Paque Premium density gradient medium (Cytiva, cat no: 17544202)
- CryoStor CS10 freezing medium (Biolife solutions, cat no: 210102)
- TheraPEAK PBS buffer (Lonza Bioscience, cat no: BEBP17-516Q)
- Human Serum Albumin

Methods:

1. Add 15 ml of Ficoll-Paque into the SepMate™ tube by carefully pipetting it through the central hole of the SepMate™ insert. The top of the density gradient medium will be above the insert.
2. Dilute the whole blood with an equal volume of PBS + 2% human serum albumin and mix gently.
3. Keeping the SepMate™ tube vertical, add the diluted whole blood by pipetting it down the side of the tube. The diluted whole blood will mix with the lymphoprep above the insert.

4. Centrifuge at 1200 x g for 10 minutes at room temperature, with the brake on.
5. Pour off the top layer, which contains the enriched PBMCs, into a new tube. Do not hold the SepMate™ tube in the inverted position for longer than 2 seconds.
6. Wash the PBMC layer with 4 volumes of PBS supplemented with 2% human serum albumin.
7. Pelletize the PBMC (600xg, 10 min, RT).
8. Resuspend in NK MACS medium.
9. Draw a sample to perform a viable cell count followed by pelletization of the PBMC (600xg, 10 min, RT).
10. Remove the supernatant for sterility test.
11. Resuspend in CS10, aliquot and freeze the PBMC at $1x10^8$ cells/ml/cryovial.

B) NK cells Enrichment and Expansion:

Materials:

- NK MACS GMP Medium (Mltenyi Biotec, catalog no. 170-076-356)
- CliniMACS CD3/CD56 complete kit (Miltenyi Biotec, cat no: 200-074-004)
- Human Serum Albumin
- IgG solution (SK Plasma)
- Sterile water for injection (B. Braun Medical Inc)
- GMP Human AB Serum (Akron Bio, catalog no: AR1010-0100)
- GMP IL-2, IL-15 and IL-18
- Soluable MICA protein (SinoBiological, cat no: 12302-H08H)
- G-Rex 100M open system Bioreactors (Wilson Wolf)
- Single use disposable Heamocytometer (Incyto, cat no: DHC-N01)
- Trypan blue solution, 0.4% (Bio-Rad, cat no. 1450021)
- CryoStor CS10 freezing medium (Biolife solutions, cat no: 210102)
- T175 culture flasks (Thermofisher Scientific, cat no: 159910)
- CryoMACS freezing bag 50 (Miltenyi Biotec, cat no: 200-074-400)
- Plasmalyte 148 solution

Method:

	Steps
Day 0	1. Frozen $15x10^9$ PBMC cells are thawed. 2. Pooled the PBMC cells in NK MACS media supplemented with 10% human serum albumin (HSA). 3. Wash once with PBS supplemented with 2% HSA. 4. Re-suspend PBMC in NK MACS media supplemented with 2% HSA before transferring into a 600ml transfer bag. 5. Collect a sample of total NK cells for Purity and Viability determination using Flow analysis. 6. Also, the purified NK cells will undergo a viable cell count to ascertain the total number of NK cells. 7. Introduce the PBMC cells into the CliniMACS Prodigy system for automated NK cells enrichment using the CliniMACS CD3/CD56 complete kit.

	8. After enrichment, 200x10^6 purified NK cells are pelletized by centrifugation (300xg, 5 min, RT) follow by resuspension in 200mL of NK Growth Medium supplemented with Human AB Serum (5%), IL-2 (200 IU/ml), MICA (50ng/ml) and cytokine cocktail (IL-15 (50ng/ml) and IL-18(250ng/ml)). 9. The re-suspended NK cells are introduced into the G-Rex100M bioreactor before placing the bioreactor into an CO_2 incubator. 10. The culture condition is to be maintained at 37°C, 5% CO_2. 11. Growing NK cells will settle at the bottom of the bioreactor to undergo proliferation.
Day 3	1. Perform a viable NK cell count. 2. Add 800mL of fresh NK MACS media supplemented with 5% human AB serum and 200IU/ml IL-2. 3. After the addition, the total culture volume is 1000mL.
When NK cells density is **≥ 1 × 10^6 cells/ml**. (typically between Days 9-12	1. NK cell count will be performed once every 3-4 days. 2. To perform a viable NK cell count, 800mL of the spent medium in the G-Rex100M Bioreactor are remove first. 3. NK cells at the bottom will be mixed in the residual 200mL of medium. 4. A sample is then taken for cell count. 5. The cell number is estimated by trypan blue exclusion method with a haemocytometer. 6. The 800mL removed in Step 2 will be put back into the G-Rex Bioreactor to enable further culture. 7. When NK cells density is ≥ 1 × 10^6 cells/ml, the 1000ml NK cell culture is equally distributed into five G-Rex100M bioreactors. 8. Additional MICA is added to a final concentration of 50ng/ml. 9. Additional IL-2 is added to a final concentration of 200IU/ml.
Three days later	1. A viable NK cells cell count is performed as outlined before. 2. Add 300ml of NK MACS media supplemented with 5% human AB serum and IL-2 (200IU/ml) to each G-Rex100M bioreactor.
Two days later	1. Perform a viable cell count as outlined before. 2. Add another 500ml of NK MACS media supplemented with 5% human AB serum and IL-2 (200IU/ml) to each G-Rex100M bioreactor to a volume of 1000ml.
After another 10-14 days when the	1. Perform a viable NK cell count as outlined before. 2. The expanded NK cells are harvested. 3. During NK cells harvesting, 40mL of spend medium is collected for Sterility test.

cell density is ≥ 2.5 × 10^6 cells/ml	4. A cell sample is collected for purity and viability determination by flow cytometry. 5. Another cell sample is collected for cytotoxicity assay to be performed in our research lab. 6. The rest of the NK cells will be labelled, aliquoted and frozen in CS10 at 5x10^8 cells/20mL in each 20ml CryoMACS freezing bag 7. The bags are cryopreserved and stored, ready to be use.

C) NK cells preparation for infusion:

Day of infusion	1. Thawed sufficient cryo-preserved NK cells for infusion. 2. Dilute the thawed NK cells with 10x volume of washing buffer (Saline solution, HSA (5%)) 3. Centrifuge (500xg, 10min,4°C) to pelletize the NK cells. 4. Remove the supernatant containing DMSO. 5. Resuspend the NK cells in infusion media (Plasma-Lyte148, HSA (2%), IL-2(200IU/ml)). 6. Take a sample to analyse the purity and viability. 7. As a risk minimizing step, if the viability falls below the range of 40% - 70%, the viability is increase through counterflow centrifugation via the Rotea system. 8. Keep the NK cells in infusion bag at 4°C (no more than 6 hr).

D) Sterility Test:

The cell culture Sterility assay will be outsourced to TUV Ltd. In this assay, the cell culture media is to be determined for the absence of the following:

- Mycoplasma
- Endotoxin
- Sterility

E) Purity and Viability Test:

The purity and viability of the expanded NK cells is determined by Flow Cytometry Analysis. In brief, approximately 1x10^6 NK cells from the expansion will be stained for CD56 (Miltenyi Biotec, cat no: 130-114-549), CD3 (Miltenyi Biotec, cat no: 130-114-710, and 7AAD Viability Dye (Miltenyi Biotec, cat no: 130-111-568). After staining, the purity and viability of NK cells can be calculated

5.3 Storage and product accountability

A. Storage:

- Allogeneic NK cells must be stored in a cryopreserved state at **≤ -150°C** (e.g., vapor phase of liquid nitrogen) to maintain viability and functionality.

- Upon thawing, cells should be used immediately or within a specified short window (typically within **6 hours** at 2–8°C) to preserve cell quality.
- Avoid repeated freeze-thaw cycles to prevent loss of viability.

B. Label Elements on the Investigator Product

- **Product Identification:** AlloNK1 – Allogenic NK cells
- **Batch/Lot number**: Unique identifier for traceability (each manufacturing run).
- **Study Protocol Number/Code**: 001
- **Statement "For Investigational Use only"**
- **Route of Administration**: For intravenous infusion only
- **Storage Conditions**: Store at 2–8 °C. Do not freeze and protect from light.
- **Date of Manufacture / Expiration**: Date of Manufacture, Date of Expiration
- **Quantity/ Volume/ Cell Dose**: Total cell number, total volume (ml) and the dosage
- **Sponsor Name & Contact**: Dr Lim Chwee Ming, (65) 98247996
- **Handling/ Special Precautions**: Handle using aseptic technique and for allogenic use only
- **Instruction of Use**: See Investigator's Brochure for administration instructions

C. Shipping:

- Cryopreserved NK cell products must be shipped in validated dry vapor shippers that maintain temperatures at or below **-150°C** throughout transit.
- Shipping containers must be equipped with temperature monitoring devices to document temperature stability during transport.
- Shipping must be coordinated to ensure timely delivery and immediate transfer to appropriate storage upon receipt.
- Shipping documentation must include product identification, batch number, storage instructions, and emergency contact information.

D. Disposal of Investigational Product on site

- Any unused, or nonconforming NK cell products must be disposed of following institutional and regulatory biosafety guidelines for biohazardous materials.
- Disposal procedures should ensure inactivation of viable cells through validated methods (e.g., autoclaving, chemical disinfection).
- All disposal activities must be documented, and records maintained in accordance with applicable regulations.

E. Disposal of <u>Expired</u> Investigational Product

- Any expired NK cell products will be transferred to our research lab to be used for preclinical investigation.

6 TREATMENTS

6.1 Rationale for Selection of Dose

In this trial, allogeneic NK cells are being used to add onto the standard chemoradiotherapy for NPC. The Maximum Tolerated Dose (MTD) will be between $0.5x10^7$ to $1.8x10^7$ NK cells/Kg (equivalent to approximately 0.5 to 1.0 billion NK cells). This dosage range of allogeneic NK cells is selected based on several Phase I cancer studies as a guide [7-10].

In these Phase I studies using allogeneic NK cells as the sole therapeutics, the dosage of allogeneic NK cells in the range of 1- 3 billions allogeneic NK cells exhibiting well tolerance. Therefore, in this Phase I trial with the infusion of allogeneic NK cells complementing standard chemoradiotherapy, we have proposed a lower dose of $0.5x10^7$ to $1.8x10^7$ NK cells/Kg (equivalent to approximately 0.5 to 1.0 billion NK cells)

6.2 Study Drug Formulations

The NK cells to be infused into NPC patients will undergo a media change to infusion media (Plasma-Lyte148, 2% human serum albumin, Interleukin-2 (200 IU/ml)) before transferred into in infusion bag and will be stored at 4°C (no more than 6 hr).

6.3 Study Drug Administration

Six doses of allogeneic NK cells at MTD will be administered for every accrued patient at during and after the radiotherapy via intravenous injection. Intravenous injection is selected because the intended target of the infused allogeneic NK cells is the small NPC cell clusters generated from the solid NPC tumour circulating in the blood during and after radiotherapy. The allogeneic NK cells infused into each patient will be derived from the PBMC of up to of healthy volunteers.

6.4 Specific Restrictions / Requirements

Routine pre-medication including steroid, antihistamine, and acetaminophen is not allowed prior to NK cell infusion.

In the event of an infusion reaction, the patient may be treated accordingly although steroids should be avoided if possible (though not absolutely contraindicated). The use of agents such as H2 antagonists, antihistamines, acetaminophen, etc. is permitted for the treatment of infusion reactions. After appropriate treatment and resolution of the infusion reaction, NK cell infusion may be resumed at the discretion of the treating physician.

6.5 Blinding

This Study is an open label Phase 1 which does not require blinding

6.6 Concomitant therapy

1. Radiotherapy will be given as per standard of care treatment.
2. Chemotherapy:
    i) Platinum based chemotherapy regime is usually administered as the standard of care chemotherapy option Platinum based chemotherapy regime is usually administered as the standard of care chemotherapy option for NPC.
    ii) In patients who cannot receive chemotherapy or declined chemotherapy option, they will still be eligible to participate in this trial.
    iii) Patients who are prescribed other chemotherapy beside the platinum-based chemotherapy option due to adverse response to platinum chemotherapy are also eligible to participate in this trial.

7 SAFETY MEASUREMENTS

7.1 Definitions

An adverse event (AE) is any untoward medical occurrence in a trial participant administered a cell, tissue and gene therapy products (CTGTP) and which does not necessarily have a causal relationship with this treatment.

An AE can therefore be any unfavourable and unintended sign (including an abnormal laboratory finding, for example), symptom, or disease temporally associated with the NK cell product.

A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

- results in death
- is life-threatening
- requires inpatient hospitalisation or prolongation of existing hospitalisation
- results in persistent or significant disability/incapacity, or
- is a congenital anomaly/birth defect

7.2 Collecting, Recording and Reporting of Serious Adverse Events (SAEs) to CIRB

The reporting requirements will be in accordance with the reporting requirements published on CIRB website at the time when the event took place.

Only related SAEs (definitely/ probably/ possibly) will be reported to CIRB. Related means there is a reasonable possibility that the event may have been caused by participation in the clinical trial.

The investigator is responsible for informing CIRB after first knowledge that the case qualifies for reporting. Follow-up information will be actively sought and submitted as it becomes available.

Related AEs will not be reported to CIRB. However, the investigator is responsible to keep record of such AEs cases at the Study Site File.

7.3 Collecting, Recording and Reporting of Serious Adverse Events (SAEs) to the Health Sciences Authority (HSA)

The investigator will assess the severity of each AE and SAE reported during the study. AEs and SAEs should be assessed and graded based upon NCI-CTCAE v5.0.

Toxicities that are not specified in NCI-CTCAE will be defined as follows:

- Grade 1: Mild; asymptomatic or mild symptoms; clinical or diagnostic observations only; intervention not indicated
- Grade 2: Moderate; minimal, local, or non-invasive intervention indicated; limiting age-appropriate instrumental activities of daily living (ADL)
- Grade 3: Severe or medically significant but not immediately life-threatening; hospitalization or prolongation of hospitalization indicated; disabling; limiting self-care ADL
- Grade 4: Life-threatening consequences; urgent intervention indicated
- Grade 5: Death related to AE

The following are **not** considered to be SAEs:

- Hospitalization for elective treatment of a preexisting condition that did not worsen from baseline
- Hospitalization for social/convenience considerations
- Scheduled therapy for the target disease of the study, including admissions for transfusion support or convenience

The assessment of study drug relationship to each AE will be reported on the appropriate source document (and SAE form, in the event of an SAE) by the Investigator or designee according to his or her best clinical judgment. The criteria listed in Table xx should be used to guide this assessment. Please note that not all criteria must be present to be indicative of a particular drug relationship.

In this study, imAEs are of special interest. Potential imAEs are listed in Table 2 below:

Table 2: Adverse Event Causality Guidelines

Body System Affected	Events
Skin (mild-common)	pruritus or maculopapular rash; vitiligo
Skin (moderate)	follicular or urticarial dermatitis; erythematous/lichenoid rash; Sweet syndrome
Skin (severe-rare)	full-thickness necrolysis/Stevens-Johnson syndrome
Gastrointestinal	colitis (includes diarrhoea with abdominal pain or endoscopic/radiographic evidence of

	inflammation); pancreatitis; hepatitis; ALT/AST elevation; bowel perforation
Endocrine	thyroiditis, hypothyroidism, hyperthyroidism; hypophysitis with features of hypopituitarism, eg, fatigue, weakness, weight gain; insulin-dependent diabetes mellitus; diabetic ketoacidosis; adrenal insufficiency
Respiratory	pneumonitis/diffuse alveolitis
Eye	episcleritis; conjunctivitis; iritis/uveitis
Musculoskeletal	arthritis; arthralgia; myalgia; myasthenic syndrome/myasthenia gravis; myositis
Blood	anemia; leukopenia; thrombocytopenia
Renal	interstitial nephritis; glomerulonephritis; acute renal failure
Cardiac	pericarditis; myocarditis; heart failure
Neurologic	encephalitis; Guillain-Barre syndrome; meningitis; meningoradiculitis; meningoencephalitis; neuropathy

Classification	**Definition**
Likely Related	There is a clear temporal association between the event and the administration of study drug, a plausible mechanism for the event to be related to the study drug and causes other than the study drug has been ruled out, and/or the event re-appeared on re-exposure to the study drug.
Probably Related	The event is a known or suspected effect of the study drug and follows a reasonable temporal sequence from the administration of study drug but cannot be readily explained by study participant or study factors, and/or the event ceases or diminishes with discontinuation of study medication
Possibly Related	There is an association between the event and the administration of the study drug and there is a plausible mechanism for the event to be related to study drug, but there may also be alternative etiology, such as characteristics of the patient's clinical status or underlying disease.
Unlikely Related	The event is unlikely/remotely possible /probably not related to the study drug and

	likely to be related to factors other than the study drug.
Not Related	The event is related to an etiology other than the study drug (the alternative etiology must be documented in the study patient's medical record).

7.4 Known or potential adverse event related to post-infusion of IP (AlloNK1)

Events that are undesirable but usually manageable or mild/moderate, without causing life-threatening risk, hospitalization, or permanent impairment such as the following:

- Infusion-related reactions (<5%): fever, chills, rigors, headache, mild hypotension.
- Mild Infections (<5%): uncomplicated bacterial/viral infection related to transient immunosuppression.
- Cytopenia (5-10%): neutropenia, anaemia, thrombocytopenia (usually CRT-related).
- Mild Hepatic/renal toxicities (<5%): Transient enzyme elevations or creatinine rise.
- Mild Neurotoxicity (<1%): mild confusion without major sequelae.

7.5 Known or potential serious adverse event related to post-infusion of IP (AlloNK1)

Events that result in death, are life-threatening, require/prolong hospitalization, cause persistent disability, or are medically significant such as the following:

- Cytokine Release Syndrome (CRS): even if rare and milder than CAR-T cells, if it causes hypotension requiring vasopressors, high-grade fever, or ICU-level care, it will be considered as an SAE.
- Severe infections: presence of sepsis, pneumonia, or other infections requiring IV antibiotics or hospitalization.
- Graft-versus-host disease (GVHD): any clinically significant GVHD is considered an SAE.
- Hepatic/renal toxicities – acute liver failure, grade 3–4 enzyme elevations, renal failure requiring dialysis.
- Neurotoxicity: seizures, encephalopathy, or any neurological event requiring intervention or hospitalization.
- Tumour lysis syndrome: especially if it leads to renal impairment, arrhythmia, or hospitalization.

7.6 Management of AE and SAE

Our team comprises of haematologists specializing in managing these cell therapy related complications indicated table 4:

Table 4

Events	Recommended Management
Infusion reaction (mild–moderate)	Pause/slow infusion, give antipyretics, antihistamines; monitor vitals closely.
CRS (grade ≥2)	Supportive care (IV fluids, oxygen), consider tocilizumab / corticosteroids as deemed appropriate by our haematologist. Escalate to HD or ICU care if hypotensive requiring vasopressors.

Infection	Prompt cultures, broad-spectrum antibiotics; prophylactic antimicrobials per institutional guidelines during neutropenia.
Cytopenia	Growth factor support (G-CSF), transfusions as indicated.
GVHD (if suspected)	Standard immunosuppressive therapy (high-dose steroids) and consult transplant/immune specialists.
Neurotoxicity	Supportive management, consider corticosteroids, seizure prophylaxis if indicated.
Tumour lysis / electrolyte abnormalities	Hydration, monitor electrolytes, rasburicase/allopurinol as per protocol.

7.7 Safety Monitoring Plan

The principal investigator will be responsible for the safety and well-being of subjects, and integrity of data collected.

a) Monitoring Requirement for NPC patient receiving IP:

Parameter	Timing/Frequency	Notes
Vital signs (temperature, blood pressure, heart rate, respiratory rate, oxygen saturation)	Before, during, and after each NK cell infusion (e.g., baseline, 15 min, 30 min, 1 h, 2 h post-infusion)	Monitor for infusion allergic reactions, hypotension, fever/chills.
Complete blood count (CBC) with differential & platelets	Baseline, then at least twice weekly during chemoradiotherapy, and prior to each NK cell infusion	Detect cytopenia from CRT
Comprehensive metabolic panel (liver enzymes, renal function, electrolytes)	Baseline, weekly or more frequently if abnormal	Monitor for hepatic/renal toxicity.
Coagulation profile (PT, aPTT, fibrinogen)	Baseline and as clinically indicated	Evaluate for coagulopathy and possible CRS or infection.
Infection screening (blood cultures, CRP/procalcitonin if febrile)	Baseline and as clinically indicated	Example for rare rick of neutropenic infection.
Immune parameters (NK cell counts, cytokine panels, EBV DNA load)	Baseline, then specified time points post-infusion	Monitor NK persistence, immune activation, EBV viral load in NPC context.

Cardiac monitoring (ECG, troponin, echocardiogram	Baseline and if any symptoms	Recommended if CRT includes cardiotoxic agents or for severe CRS
Neurological checks	Daily during hospitalization; at each visit outpatient	Watch for neurotoxicity (rare).

b) Data and Safety Monitoring

A safety review committee (SRC) will monitor the safety data during Phase 1 of the study on a periodic basis to help ensure the ongoing safety of study patients. The SRC will consist of at least 3 investigators of the study, a pharmacist and biostatistician. The SRC will meet regularly during the Phase 1 dose escalation portion of the study to review all accumulated available data, including safety and preliminary efficacy to make recommendations on continuation, modification, or suspension of the study. The SRC will make recommendations concerning dose escalation/de-escalation or any changes to the dosing paradigm based on review of available data prior to proceeding to the next dose level. The SRC will also make recommendations about the dose selected for Phase 2 and overall study conduct.

7.8 Complaint Handling

Any compliant will be submitted to the SRC for their review process. A response will be provided within 21 days.

8 DATA ANALYSIS

8.1 Data Quality Assurance

Data will be collected and recorded by a clinical research coordinator. The investigator will verify that all data entries are accurate and correct, including verification that the subject fulfils the criteria for entry into the study before study treatment commences.

8.2 Data Entry and Storage

All data obtained in the study described in this protocol will be recorded on paper case report forms (CRFs). The CRF for each subject will be presented in a folder. The CRF will be completed chronologically and updated regularly in order to reflect the most recent data on the patient included in the study.

Each CRF must be neatly filled in with a black-inked pen. For each page on which information is entered, the subject number must be recorded. CRFs will be dated and signed by an investigator.

Errors must be corrected by drawing a single line through the incorrect entry and by writing the new value as close as possible to the original. The correction must then be initialed and dated by an authorized study team member.

CRFs and Investigator Site Folder will be kept in a secure cabinet, under lock and key. Only members of the study team can have access to the data.

9 SAMPLE SIZE AND STATISTICAL METHODS

9.1 Determination of Sample Size

In the Phase I study, we will be using the Bayesian Model Averaging continual reassessment method (BMA-CRM) study design [12, 13]. The primary goal of the BMA-CRM is to identify the maximum tolerated dose (MTD) of NK cells to be infused. In this Phase I study, minimum of 21 patients will be accrued. Five doses of NK cells will be used ($0.5x10^7$, $0.8x10^7$, $1.1x10^7$, $1.4x10^7$ and $1.8x\ 10^7$cells/Kg). We will begin with the dose $0.8x10^7$cells/kg and the dose escalation is restricted to a single dose level. Once there is an occurrence of 1 toxicity, the CRM will be implemented. The CRM method estimates toxicity at each dose using Bayesian methodology to update a dose-toxicity curve for each cohort of 3 patients. We assume the prior toxicity probability estimates at 10%, 15%, 20%, and 25% at each level. To avoid subjectivity in setting priors, the BMA-CRM specifies multiple sets of priors and takes the average across the CRM models to avoid the potential estimation bias caused by misspecification. We shall terminate the phase at 21 patients or when CRM dictate that 6 patients be treated at a particular dose, at which point the current dose estimate is declared the MTD.

Phase 2 is a single-arm trial; the one-sample proportion test will be used to test the proportion deduction based on the binomial distribution. A sample size of 5 achieves 80.549% power to detect a difference (P1-P0) of -0.2500 using a one-sided exact test with a significance level (alpha) of 0.05. These results assume that the population proportion under the null hypothesis (P0) is 1.00. Assume the withdrawal rate at 0.2 (20%), the total sample size is estimated at 6. From phase 1a, for those patients treated at MTD, these patients will be followed up and included in the phase 2 study. As likely there will be around six patients treated at MTD (at least two cohorts), thus only an additional one patient will be included for the phase 2 trial.

9.2 Statistical and Analytical Plans

a. General Considerations

The phase 1 study will be an open-label, dose-escalation trial, with a maximum of 21 NPC patients to be accrued for NK cells treatment. The primary endpoint is the occurrence of any of the toxicities during 4 weeks of treatment as assessed by AEs attributed (see session 7.3). Five doses of NK cells will be used for the treatment ($d_1 = 0.5x10^7$, $d_2 = 0.8x10^7$, $d_3 = 1.1x10^7$, $d_4 = 1.4x10^7$, and $d_5 = 1.8x10^7$ cells/kg). The maximum tolerated dose (MTD) will be estimated as the highest dose with an estimated probability of a dose limiting toxicity (DLT) of less than 30%.

We will apply 2-stage continual reassessment method (CRM) design (Cheung 2005 & 2011; Goodman 1995). First, we specify a stage 1 design as a predetermined nondecreasing dose sequence. Because the 3+3 algorithm is familiar, we consider the “group-of-three” stage 1 design whereas escalation takes place after every group of three nontoxic outcomes. The stage 1 design is in effect until the first observed toxicity. Secondly, the trial turns to the model-based CRM for dose assignment. CRM method generally has a superior operating

performance as it bases estimation on all prior dose data rather than just the last 3 patients; this usually resulted in more accurate estimation of the MTD, fewer toxicities and more patients treated at assumed efficacious doses close to the MTD.

Specifically, for stage 1:
The first three patients ($x_{i,0}$) will be recruited at dose level d_2. Starting at d_2 is because that NK cell treatments are given at established doses which is thought to be non-toxic.

If no DLT occurs, subsequent three patients will be recruited at one dose higher (unless current dose is D6).

$x_{1,0} = x_{2,0} = x_{3,0} = d_2$, $x_{4,0} = x_{5,0} = x_{6,0} = d_3$,. . .

The MTD will be estimated as the last dose (d_5).

If a DLT occurs, the trial will progress to stage 2 using a CRM to assign the dose (see below).

For stage 2:

The study will progress to this stage if a DLT occurs in stage 1. We will assign 3 patients at a time to each does and update the model-based estimates between every group of 3 patients, using information of all patients with DLTs known by the next patient recruitment time. Escalation by more than 1 level will not be allowed. If the number of consecutive patients on the current dose is 6 or 21 patients in total, then the trial will stop and the MTD will be estimated based on a CRM of the recruited patients.

We use maximum likelihood estimation in conjunction with the CRM. The idea is analogous to the Bayesian CRM, with the data observed in the first $i-1$ patients. The dose for the next subject is computed based on the maximum likelihood estimates of coefficients for given data.

A CRM will be implemented via the function implemented in R package “dfcrm” [15]. The prior toxicity estimates are set at 2.5%, 5%, 10%, 20%, 30% for the dose levels from D1 to D6. We shall terminate the phase at 21 patients or when a dose level is selected 6 times at which point the current dose estimate is declared the MTD.

The properties of the CRM procedure are assessed using the CMR Suite v1.00 using simulated data [12, 13]. We assume patients recruited randomly, with average arrival of 1.25 per month, toxicity assessment period is within 30 days, and target toxicity limit (TTL) probability is 30%. The simulation is applied to 5 scenarios from true low to very high toxicity rates using 1000 replicates for each scenario. The table below compares the performance in each circumstance in terms of the total and an average number of DLT, the proportion of times each dose level is selected as MTD, the proportion of total patients treated at each dose level, and the duration of trial time. If the true toxicity is low, around 19.8 months are expected to complete the trial, and the toxicity per trial is low at 2.7, and MTD is estimated at the dose level 5.

Model

Target Toxicity Probability	0.300

Safety Stopping Probability (Upper Limit Pr(tox) at Lowest Dose)	0.700
Maximum Sample Size	21
Cohort Size	3
Starting Dose Level	2
Toxicity Assessment Period (Days)	30

Prior **Median** Probabilities of Toxicity at Each Dose Level:

Dose	Prob.
1	0.025
2	0.050
3	0.100
4	0.200
5	0.300

Scenarios and Results

Scenario: Very High Toxicity

Dose Level	True Prob(tox)	Selection Probability	# of Subjects Treated	# of Toxicities
1	0.200	0.11	1.7	0.4
2	**0.300**	0.24	7.3	2.2
3	0.400	0.22	4.6	1.8
4	0.500	0.07	1.5	0.7
5	0.600	0.01	0.2	0.1
Probability of Early Termination: 0.35				
Toxicities per Trial: 5.2				
Total Trial Time (Months): 15.65				

Scenario: High Toxicity

Dose Level	True Prob(tox)	Selection Probability	# of Subjects Treated	# of Toxicities
1	0.100	0.03	0.8	0.1
2	0.200	0.17	6.7	1.4
3	**0.300**	0.35	6.5	1.9

4	0.400	0.25	3.6	1.4
5	0.500	0.05	0.8	0.4
Probability of Early Termination: 0.16				
Toxicities per Trial: 5.2				
Total Trial Time (Months): 19.01				

Scenario: Medium Toxicity

Dose Level	True Prob(tox)	Selection Probability	# of Subjects Treated	# of Toxicities
1	0.050	0.00	0.1	0.0
2	0.100	0.03	4.6	0.5
4	0.250	0.32	5.2	1.3
5	**0.300**	0.41	4.2	1.3
Probability of Early Termination: 0.04				
Toxicities per Trial: 4.2				
Total Trial Time (Months): 20.55				

Scenario: Low Toxicity

Dose Level	True Prob(tox)	Selection Probability	# of Subjects Treated	# of Toxicities
1	0.000	0.00	0.0	0.0
2	0.025	0.00	3.2	0.1
3	0.050	0.00	3.5	0.2
4	0.100	0.06	4.3	0.4
5	**0.200**	0.93	9.9	2.0
Probability of Early Termination: 0.01				
Toxicities per Trial: 2.7				
Total Trial Time (Months): 19.85				

Phase 2: Statistical Analysis and Power calculation (new to 10%)

The reference we use in comparison with our single arm trial finding is derived from the anticipated proportion of locally advanced NPC patients (25-30%) who will continue to present with detectable circulating EBV-DNA following standard of care CRT paradigm. We hypothesize that in this single arm trial, adding allogeneic NK cells will completely eliminate the proportion of patients with detectable circulating EBV-DNA from 25-30% to 10%. Hence, applying the one proportion Z-score test, a sample size of 26 will achieve 81.2% power to detect a difference (P1-P0) of 20% using a one-sided exact test with a significance level (alpha) of 0.05. Assuming the withdrawal rate at 0.2 (20%), the total

sample size is estimated at 31. In our phase 1 design, at least 6 patients will be treated at our MTD of allogenic NK cells, meaning that additional 25 patients will be accrued in the phase 2 trial.

The proportion estimate and its confidence interval will also be reported. We will also perform sensitivity analyses to assess the impact of missing data and potential confounding variables.

The secondary endpoint of the study is to determine the change in patient survival and recurrence rate within 24-36 months post-treatment when compared with historic cohorts under standard treatments. This is an exploratory aim due to small sample size. We will apply Kaplan-Meier curves to plot the survival and recurrence rate with time. We will estimate the median and mean survival time, and the confidence intervals. The median survival time will be estimated. We will compare the survival and recurrence rates between the study cohort and the historical control group using log-rank tests.

b. Safety Analyses

The phase 1 study is safety-oriented design. Adverse events will be monitored and recorded throughout the study. Dose toxicity evaluation after receiving the treatment will be conducted. Dose escalation would occur only if no toxicity occurred or as suggested by CRM. Dose-limiting toxicities will be defined and recorded. We will also examine the proportion of patients who experience treatment-related adverse events and report them separately.

9.3 Interim Analyses

The dose-escalation design CRM foresees that decisions based on the current data are taken before the end of the study. More precisely, after each cohort in the dose-escalation part, the next dose will be chosen depending on the observed data. The study may be stopped early if predefined stopping rules are met.

At the mid-point of Phase 2, we will access the progress of our primary endpoint by the determination of the percentage of patients achieving undetectable EBV-DNA levels and secondary endpoint of recurrent rate among all the accrual for the trial thus far.

9.4 Describe the types of statistical interim analyses, including their timing

1. See the analysis method session for CRM methods.
2. A statistical two tailed test will be performed to examine the decrease in the percentage of patients with undetectable EBV-DNA levels,
3. Similar statistical analysis will be carried out to analyse the NPC recurrence rate among the accruals for the trial at the point of analysis.

10 DIRECT ACCESS TO SOURCE DATA/DOCUMENTS

The investigator(s)/institution(s) will permit study-related monitoring, audits and/or IRB review and regulatory inspection(s), providing direct access to source data/document.

In accordance with ICH GCP guidelines, the study monitor must have direct access to the investigator's source documentation in order to verify the data recorded in the investigator file for consistency. The monitor is responsible for routine review of the investigator file at regular intervals throughout the study to verify adherence to the protocol and the completeness, consistency, and accuracy of the data being entered on them. The investigator agrees to cooperate with the monitor to ensure that any problems detected during these monitoring visits are resolved.

11 QUALITY CONTROL AND QUALITY ASSURANCE

Data will be collected and recorded by a clinical research coordinator. The investigator will verify that all data entries are accurate and correct, including verification that the subject fulfils the criteria for entry into the study before study treatment commences.

12 ETHICAL CONSIDERATIONS

This study will be conducted in accordance with the ethical principles that have their origin in the Declaration of Helsinki and that are consistent with the Good Clinical Practice and the applicable regulatory requirements.

This final Clinical Trial Protocol, including the final version of the Participant Information Sheet and Consent Form, must be approved in writing by the Centralised Institutional Review Board (CIRB) and regulatory approval from Health Sciences Authority (HSA), prior to enrolment of any patient into the study.

The principal investigator is responsible for informing the CIRB and HSA of any amendments to the protocol or other study-related documents, as per local requirement.

12.1 Informed Consent

The study will be conducted in accordance with the protocol, International Conference on Harmonization (ICH) GCP guidelines, applicable regulations and guidelines governing clinical study conduct and ethical principles that have their origin in the Declaration of Helsinki. The investigator will ensure that the study is conducted in accordance with the provisions as stated in the ICH regulations and complies with prevailing local laws and customs. Prior to the initiation of any screening or study-specific procedures, the investigator and/or his/her representative will explain the nature of the study to the subject and answer all questions regarding this study. Each informed consent will be reviewed, signed and dated by the subject and the person who administered the informed consent. A copy of each informed consent will be given to the subject, and each original will be placed in the Investigator Site Manual.

12.2 Confidentiality of Data and Patient Records

Information collected for this study will be kept confidential. This confidentiality is extended to cover testing of any biological samples and genetic tests in addition to the clinical information relating to the participating subjects. Patient records, to the extent of the applicable laws and regulations, will not be made publicly available. Data collected and entered into the Case Report Forms are the property of SGH. In the event of any publication regarding this study, patient identity will remain confidential.

13 PUBLICATIONS

The investigators have the right to publish the results of the study, but with due regard to the protection of confidential information.

14 RETENTIONS OF TRIAL DOCUMENTS

Records for all participants, including CRFs, all source documentation (containing evidence to study eligibility, history and physical findings, laboratory data, results of consultations, etc) as well as DSRB records and other regulatory documentation will be retained by the PI in a secure storage facility for up to 10 years from time of study termination. The records will be accessible for inspection and copying by authorized authorities.

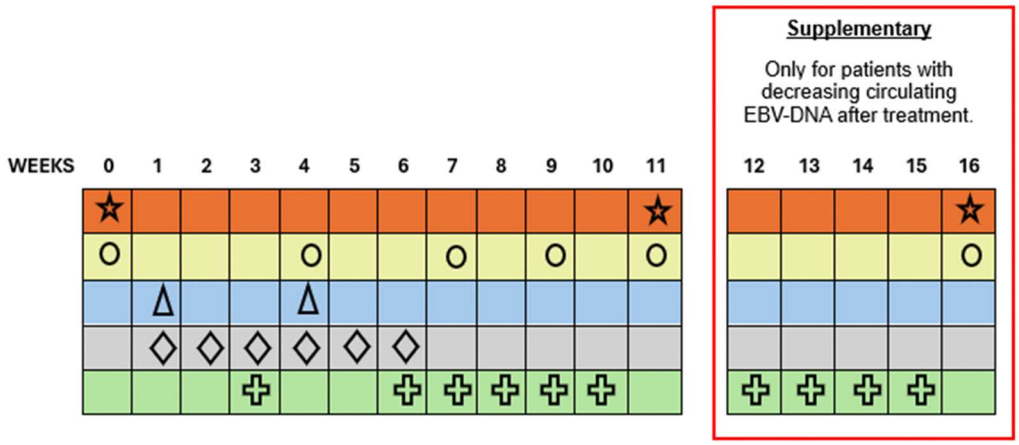
15 FUNDING AND INSURANCE

The Insurance is covered under the Singhealth Insurance policy for clinical trial in its terms and provisions, its legal liability for injuries caused to participating persons and arising out of this research performed strictly in accordance with the scientific protocol as well as with applicable law and professional standards. Since this is a first in man clinical trial, additional insurance will be acquired.

The funding is supported under the PI’s National Medical Research Council. Clinician Scientist Award (CSA) and Clinical Trial Grant – Investigator Initiated trial (CTG-IIT).

16 LIST OF ATTACHMENTS

A) Appendix 1 Study Schedule



Legend

☆	Collection of Patient Tissue Biopsy Sample for Review
O	Collection of Patient Blood Sample for Review
Δ	Chemotherapy at day 1 of the week
◇	Radiotherapy (5 doses/week)
✚	Allogenenic NK cell infusion (1 dose/week)

Assessment Period	Assessment Procedures	Notes
-28 to -1	1. Draw Blood (20ml) 2. Tumour Tissue Biopsy 3. EBV assay	
Week 1	1. Chemo-drug Administration 2. Radiation Therapy Administration (1 dose daily, 5 doses/week)	
Week 2	1. Radiation Therapy Administration (1 dose daily, 5 doses/week)	
Week 3	1. Radiation Therapy Administration (1 dose daily, 5 doses/week) 2. NK cells infusion	
Week 4	1. Draw Blood (20ml)* 2. EBV assay 3. Chemo-drug Administration 4. Radiation Therapy Administration	*Draw blood before Chemo-drug administration

	(1 dose daily, 5 doses/week)	
Week 5	1. Radiation Therapy Administration (1 dose daily, 5 doses/week)	
Week 6	1. Radiation Therapy Administration (1 dose daily, 5 doses/week) 2. NK cells infusion	
Week 7	1. Draw Blood (20ml) * 2. EBV assay 3. NK cells infusion	*Draw blood before NK cells infusion
Week 8	1. NK cells infusion	
Week 9	1. NK cells infusion	
Week 10	1. NK cells infusion	
Week 11	1. Draw Blood (20ml) 2. Tumour Tissue Biopsy 3. EBV assay	
Week 12 (Optional)	1. NK cells infusion	
Week 13 (Optional)	1. NK cells infusion	
Week 14 (Optional)	1. NK cells infusion	
Week 15 (Optional)	1. NK cells infusion	
Week 16 (Optional)	1. Draw Blood (20ml) 2. Tumour Tissue Biopsy 3. EBV assay	

B) <u>NK cell infusion</u>

1. 6 doses will be administered during the standard of care treatment of concurrent chemoradiotherapy (2 doses as concurrent and 4 doses as adjuvant therapy).
2. The first 3 patients admitted for the administration of the first dose of NK cells will be monitored overnight. The expected duration of NK cell infusion is approximately 30-60 mins over a slow infusion. If there are no acute reactions to NK cell infusion for the first 3 patients, subsequent doses to the 3 patients can be treated as outpatient with a monitoring of 4-6 hours following infusion.
3. Without acute reaction, NK cells infusion for the rest of the patients accrued will be treated as outpatient with a monitoring of 4-6 hours following infusion
4. Patients who have persistently detectable circulating plasma EBV-DNA copies after the 6 doses of NK cell infusions will have the option of receiving an additional 4 weekly cycles of NK cells infusion.

C) <u>Radiation Therapy</u>

Conventional radiotherapy will be given as per standard of care treatment of NPC

D) Chemotherapy

Platinum based chemotherapy regime is usually administered as the standard of care chemotherapy option for NPC. In patients who cannot receive chemotherapy or declined chemotherapy option, they will still be eligible to participate in this trial. Patients who are prescribed other chemotherapy beside the platinum-based chemotherapy option due to adverse response to platinum chemotherapy are also eligible to participate in this trial.

E) Correlative tissue Biopsy and blood analyses

1. A total of 2 tumour biopsies will be performed in this trial (once prior to treatment and the other after the completion of the standard treatment and 6 doses of NK cells infusion).
2. Blood (approximately 20 ml) will be drawn 5 times (prior to treatment, week 4, 7, 9 and 11) during the trial. Sufficient plasma will be sent for plasma EBV detection, and the rest will be frozen for analysis at a later stage.
3. PBMC will be isolated and stored for correlative analyses.

17 REFERENCES

1. Del Zotto, G., et al., *Markers and function of human NK cells in normal and pathological conditions.* Cytometry B Clin Cytom, 2017. **92**(2): p. 100-114.
2. Wu, J. and L.L. Lanier, *Natural killer cells and cancer.* Adv Cancer Res, 2003. **90**: p. 127-56.
3. Dianat-Moghadam, H., et al., *NK cells-directed therapies target circulating tumor cells and metastasis.* Cancer Lett, 2021. **497**: p. 41-53.
4. Han, B., et al., *Altered NKp30, NKp46, NKG2D, and DNAM-1 Expression on Circulating NK Cells Is Associated with Tumor Progression in Human Gastric Cancer.* J Immunol Res, 2018. **2018**: p. 6248590.
5. Myers, J.A. and J.S. Miller, *Exploring the NK cell platform for cancer immunotherapy.* Nat Rev Clin Oncol, 2021. **18**(2): p. 85-100.
6. Shimasaki, N., A. Jain, and D. Campana, *NK cells for cancer immunotherapy.* Nat Rev Drug Discov, 2020. **19**(3): p. 200-218.
7. Ciurea, S.O., et al., *Phase 1 clinical trial using mbIL21 ex vivo-expanded donor-derived NK cells after haploidentical transplantation.* Blood, 2017. **130**(16): p. 1857-1868.
8. Hoogstad-van Evert, J., et al., *Intraperitoneal infusion of ex vivo-cultured allogeneic NK cells in recurrent ovarian carcinoma patients (a phase I study).* Medicine (Baltimore), 2019. **98**(5): p. e14290.
9. Ishikawa, T., et al., *Phase I clinical trial of adoptive transfer of expanded natural killer cells in combination with IgG1 antibody in patients with gastric or colorectal cancer.* Int J Cancer, 2018. **142**(12): p. 2599-2609.
10. Lin, M., et al., *Pembrolizumab plus allogeneic NK cells in advanced non-small cell lung cancer patients.* J Clin Invest, 2020. **130**(5): p. 2560-2569.
11. Jin, Y.Y., et al., *Chemoradiotherapy combined with NK cell transfer in a patient with recurrent and metastatic nasopharyngeal carcinoma inducing long-term tumor control: A case report.* Medicine (Baltimore), 2020. **99**(43): p. e22785.
12. O'Quigley, J., M. Pepe, and L. Fisher, *Continual reassessment method: a practical design for phase 1 clinical trials in cancer.* Biometrics, 1990. **46**(1): p. 33-48.

13. Yin, G. and Y. Yuan, *Bayesian model averaging continual reassessment method in phase I clinical trials.* Journal of the American Statistical Association, 2009. **104**(487): p. 954-968.
14. cheung, Y.K., *Coherence principles in dose-finding studies.* Biometrika, 2005. **92**: p. 10.
15. Cheung, Y.K., *Dose FInding by the Continual Reassessment Method*. 2011: Chapman & Hall/CRC press.
16. Goodman, S.N., M.L. Zahurak, and S. Piantadosi, *Some practical improvements in the continual reassessment method for phase I studies.* Stat Med, 1995. **14**(11): p. 1149-61.