RESEARCH PROTOCOL

Immunity to liver-stage *Plasmodium falciparum* in peripheral and tissue-resident immune cells (LYTIC)

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PROTOCOL TITLE

'Immunity to liver-stage *Plasmodium falciparum* in peripheral and tissue-resident immune cells'

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Principal investigator/project leader	Matthew B.B. McCall, MD PhD Department of Medical Microbiology 268 Radboudumc P.O. Box 9101 6500 HB Nijmegen The Netherlands Tel: +31 (0)24 3619515 Email: matthew.mccall@radboudumc.nl
Sponsor (in Dutch: verrichter/opdrachtgever)	Radboudumc Department of Medical Microbiology Represented by: Prof. Heiman Wertheim, MD PhD (HoD) Tel: +31 (0)24 3619041 E-mail: heiman.wertheim@radboudumc.nl
Clinical supervisors	Prof. Hans de Wilt, MD PhD Department of Surgery Radboudumc Tel : +31 (0)24 3617365 E-mail: Hans.deWilt@radboudumc.nl Meta Roestenberg, MD PhD

	Department of Medical Microbiology 268 Radboudumc Tel: +31 (0)24 3619515 E-mail: meta.roestenberg@radboudumc.nl
Trial nurse	Linda Garms Department of Surgery Radboudumc Tel : +31 (0)24 36193832 E-mail: Linda.Garms@radboudumc.nl
Subsidising party	***N/A***
Independent expert (s)	Dr. Andre J. A. Bremers, MD, PhD Department of Surgery Tel: +31 (0)24 3668086 E-mail: Andre.Bremers@radboudumc.nl
Immunological investigator	Xi Zen Yap, PhD Department of Medical Microbiology 268 Radboudumc Tel: +31 (0)24 3614664 E-mail: zen.yap@radboudumc.nl

PROTOCOL SIGNATURE SHEET

Name	**Signature**	**Date**
Sponsor or legal representative: **Head of Department:** Prof. Heiman Wertheim, MD PhD (HoD) Department of Medical Microbiology		
Principal Investigator: **Matthew B.B. McCall, MD PhD** Department of Medical Microbiology Radboudumc		

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LIST OF ABBREVIATIONS AND RELEVANT DEFINITIONS

AE	**Adverse Event**
ANOVA	**Analysis of Variance**
AR	**Adverse Reaction**
CA	**Competent Authority**
CCMO	**Central Committee on Research Involving Human Subjects; in Dutch: Centrale Commissie Mensgebonden Onderzoek**
EMA	**European Medical Agency**
FACS	**Flow-Assisted Cell Sorting**
GCP	**Good Clinical Practice**
GDPR	**General Data Protection Regulation; in Dutch: Algemene Verordening Gegevensbescherming (AVG)**
HBV	**Hepatitis B Virus**
HCV	**Hepatitis C Virus**
HIV	**Human Immunodeficiency Virus**
IB	**Investigator's Brochure**
IC	**Informed Consent**
IFN-γ	**Interferon gamma**
MACS	**Magnet-activated cell sorting**
METC	**Medical research ethics committee (MREC); in Dutch: medisch-ethische toetsingscommissie (METC)**
NK cell	**Natural Killer cell**
PBMC	**Peripheral Blood Mononuclear Cell**
(S)AE	**(Serious) Adverse Event**
Sponsor	**The sponsor is the party that commissions the organisation or performance of the research, for example a pharmaceutical company, academic hospital, scientific organisation or investigator. A party that provides funding for a study but does not commission it is not regarded as the sponsor, but referred to as a subsidising party.**
SUSAR	**Suspected Unexpected Serious Adverse Reaction**
UAVG	**Dutch Act on Implementation of the General Data Protection Regulation; in Dutch: Uitvoeringswet AVG**
WMO	**Medical Research Involving Human Subjects Act; in Dutch: Wet Medisch-wetenschappelijk Onderzoek met Mensen**

SUMMARY

Rationale: Malaria is a major cause of mortality in endemic regions. The malaria parasite *P. falciparum* has an initial asymptomatic life stage within host hepatocytes (liver-stage) which if disrupted prevents progression into the pathogenic blood-stage. As a result, the liver-stage is a target for many current malaria vaccine candidates, but immunity against this cryptic stage is not well understood.

Objective: In this study we will establish an *in vitro* assay using leukocytes (CD8+ T cell line, fresh PBMCs and liver-resident lymphocytes) in combination with freshly isolated human hepatocytes to study respectively cytolytic T cell and innate immune recognition and killing of *P. falciparum*-infected hepatocytes..

Study design: This is a single-centre investigator-initiated exploratory study where immune cells and hepatocytes will be isolated from blood and liver sections of patients at the Radboudumc undergoing medically-indicated liver surgery. Prior to surgery, written informed consent will be obtained and in total 30mL of blood will be drawn to identify donors with the HLA-A2 phenotype and for isolation of immune cells.

Study population: Patients (M/F) over 18 years of age undergoing medically-indicated partial liver resection for underlying disease.

Primary study endpoints:

- Establishment of an *in vitro* assay to study recognition and killing of *P. falciparum*-infected hepatocytes by:
    - cytolytic CD8+ T cells
    - hepatic and peripheral innate/innate-like lymphocytes

Nature and extent of the burden and risks associated with participation, benefit and group relatedness:

Blood will be collected from participants twice: 6mL will be drawn by venepuncture upon receipt of informed consent for determination of HLA-A2 phenotype, and 24mL immediately prior to surgery, via an existing intravenous or arterial line. Liver tissue, obtained from these patients undergoing medically-indicated partial liver-resection for underlying disease, that is not required for diagnostic purposes and would otherwise be considered medical waste, will be processed to obtain hepatocytes and liver-resident immune cells. The risks associated with phlebotomy are minor and there is no additional risk to patients associated with processing of already-resected liver tissue. No study procedures will interfere with routine clinical care for the participants' underlying disease. There is no direct benefit to participants for participation.

1. INTRODUCTION AND RATIONALE

Malaria caused by the Apicomplexan parasite *Plasmodium falciparum* poses a huge burden to public health in endemic regions, particularly among children and pregnant women. The parasite has a complex life cycle beginning with injection of infectious sporozoite stages into the skin through the bites of infectious mosquitoes. Sporozoites then migrate to the liver and develop within hepatocytes for ~6-7 days, during which they are asymptomatic for the host, before emerging to cause the pathogenic blood-stage of the disease. Mounting sterile immunity to liver-stage malaria parasites results in abrogation of further pathology, and has therefore served as the basis for many vaccine candidates.

However, even the single liver-stage vaccine to have currently receive European Medicines Agency approval achieves only 17-24% efficacy over seven years (1), mirroring failures of natural exposure to generate sterilely protective liver-stage immunity in the field. However, it is possible to induce liver-stage immunity through alternate immunisation strategies: immunisation using *P. falciparum* sporozoites under chemoprophylaxis (CPS) is able to generate durable sterile protection in 100% of malaria-naïve vaccinees (2,3), although this does not represent a practical approach to mass-vaccination. Immunity to liver-stage parasites in humans is still poorly understood, in large part due to limitations obtaining tissue samples of this cryptic stage in malaria patients. A better understanding thereof is urgently required to guide the rational design of next-generation liver-stage malaria vaccines.

We have recently developed an *in vitro* model of full *P. falciparum* liver-stage parasite development using freshly isolated human hepatocytes obtained from liver sections of patients undergoing medically-indicated surgery. This model has been successfully used by us to study aspects of liver-stage biology and in this respect results in significantly higher infectivity rates than models using either cryopreserved hepatocytes or hepatocyte cell lines [refs]. We have moreover been able to isolate and study the phenotype and function of liver-resident immune cells from these same liver sections (J. Walk, LIMIT study, unpublished data, accession number 2016-3049).

In murine and human studies, cytolytic $CD8^+$ T cells are important contributors to sterile immunity (4–7). While the antigenic targets of most cytolytic T cells generated by natural infection are unclear, the circumsporozoite protein (CSP) is one of these natural targets (8). We have access to an HLA-2A-restricted CSP-specific cytolytic T cell clonal line capable of lysing cells which have been artificially loaded with CSP (9). However, it remains to be demonstrated whether these cells are capable of recognising and killing *P. falciparum*-infected hepatocytes. Demonstrating functional activity of this T cell line against infected hepatocytes would provide a platform to investigate a wide range of further highly-relevant questions including immunity to genetically distinct *P. falciparum* strains (10,11), whether the parasite elicits immune recognition early or late during liver-stage development, and which immune pathways are required for killing of liver-stage parasites.

The contribution of other cells to liver antimalarial immunity is even less well understood. The liver microenvironment is widely indicated to be tolerogenic (12–14), but is nonetheless enriched in innate immune cells, particularly natural killer (NK) and innate-like γδT cells (15). Both NK cells and γδT cells have in murine studies been indicated to have a role in antimalarial protection, in part due to their high production of IFNγ (16,17). It is unclear how these cells effect liver-stage immunity, and it is also unclear whether circulatory or liver-resident innate(-like) lymphocytes differ in their ability to clear *P. falciparum* infection.

In this study we will use the *in vitro* fresh human hepatocyte model of *P. falciparum* liver-stage infection in combination with both the CSP-specific cytolytic T cell clone and donors' own lymphocytes from peripheral blood and liver to investigate whether human immune cells are able to mount functional responses to liver-resident *P. falciparum* parasites.

2. OBJECTIVES

Primary Objectives

- To establish an *in vitro* assay to study recognition and killing of *P. falciparum*-infected hepatocytes by:
    - CSP-specific cytolytic CD8+ T cells
    - hepatic and peripheral innate/innate-like lymphocytes

Secondary Objectives:

- To assess recognition and killing of *P. falciparum*-infected hepatocytes by CSP-specific cytolytic CD8+ T cell line
- To assess the differences in recognition and killing of *P. falciparum*-infected hepatocytes between liver-resident and peripheral lymphocytes
- To identify the individual lymphocyte (sub-)populations which contribute to recognition and killing of *P. falciparum*-infected hepatocytes

Exploratory Objectives:

- To assess at which time point during intra-hepatocytic development (early, middle or late) *P. falciparum*-infected hepatocytes are most optimally recognised and killed
- To determine difference in recognition and killing of *P. falciparum*-infected hepatocytes between parasite strains
- To compare recognition and killing of *P. falciparum*-infected hepatocytes in different zonal hepatocyte types
- To characterise immunological pathways involved in recognition and killing of *P. falciparum*-infected hepatocytes by lymphocyte (sub-)populations

3. STUDY DESIGN

This is a single-centre investigator-initiated exploratory study. Participants will be recruited building upon an existing collaboration with the Department of Surgery, through which we routinely receive anonymized liver tissue which would otherwise be considered medical waste, for *in vitro P. falciparum* culture from patients undergoing medically-indicated partial liver-resection for underlying disease. For the current study, during an initial visit potential participants will be provided with the information sheet and written informed consent will be obtained to draw 6mL of blood to determine if the participant expresses the HLA-A2 phenotype compatible with the CSP-specific cytolytic CD8+ T cell line. Immediately prior to surgery, an additional 24mL of blood will be drawn via an existing intravenous or arterial line for isolation of peripheral blood mononuclear cells (PBMCs). No study procedures will interfere with routine clinical care for the participants' underlying disease.
Hepatocyes will be isolated from part of the available liver tissue for *in vitro P. falciparum* culture and innate/innate-like lymphocytes will be isolated from the remaining liver tissue and PBMCs. In participants who express HLA-A2, *in vitro* hepatocyte cultures will be used to assess recognition and killing of intra-hepatocytic parasites by the CSP-specific cytolytic CD8+ T cell line. In all subjects with sufficient material, regardless of HLA-A2 expression, we will assess recognition and killing by liver-resident and peripheral innate(-like) lymphocytes. Read-out will be by variety of immunological techniques including, immunofluorescence microscopy, flow cytometry, qPCR and ELISA/multiplex bead array.

4. STUDY POPULATION

- **Population (base)**

  Adult (M/F) patients undergoing medically-indicated partial liver resection.

- **Inclusion criteria**

  In order to be eligible to participate in this study, a subject must meet all of the following criteria:

  - Scheduled for partial liver resection for underlying disease
  - Signed written informed consent

- **Exclusion criteria**

  A potential subject who meets any of the following criteria will be excluded from participation in this study:

  - Known receipt of immunosuppressive or cytostatic agents within the past 3 months, except the use of topical and inhaled steroids
  - Known human immunodeficiency virus (HIV), hepatitis C virus (HCV), or hepatitis B virus (HBV) infection; or other known clinically-relevant immunodeficient state.

- **Sample size calculation**

  No formal sample size calculation is applicable. Nevertheless, inclusion of up to 45 subjects is expected to be required to achieve all research objectives, as follows: the CD8 T cell line with which anti-parasite activity will be assessed specifically recognizes (*P. falciparum*-infected hepatocytes with) the HLA-A2 phenotype. HLA-A2 is expressed in 36-52% of the population, such that in a cohort of 45, 16 to 23 volunteers are expected to be HLA-A2$^+$. Due to the limited shelf life of fresh hepatocytes, in principle it is possible to perform a maximum of one experiment per liver donor. Although every attempt will be made, sometimes hepatocytes cannot be successfully isolated from liver donations due to surgical or biological issues.

  A minimum of five experiments will be required to optimise the protocol for killing infected hepatocytes using the CD8$^+$ T cell line. A minimum of three experiments will be required to record and quantify killing of infected hepatocytes by the CD8$^+$ T cell line and an additional three to six experiments will be needed to assess the influence of parasite development stage, *P. falciparum* strain, and hepatocyte type. These experiments will have to be performed sequentially, not simultaneously, as their results will inform the design of the next set of experiments. Depending on the

number of hepatocytes that can be collected from each volunteer, some measurements may even need to be divided across different donors. It is thus anticipated that inclusion of up to 45 participants (which is estimated to take ~1.5 years) is required to attain the primary and secondary study objectives. If all research objectives are achieved before all 45 volunteers have been recruited, recruitment will be terminated early.

5. TREATMENT OF SUBJECTS

For this study, blood will be drawn twice from participants: 6mL peripheral venous blood at Inclusion and a further 24mL of whole blood through an existing intravenous or arterial line immediately prior to surgery.

Patients will undergo medically-indicated routine partial liver resection and all other routine clinical care for their underlying disease, which falls outside of the context of this exploratory study.

6. INVESTIGATIONAL PRODUCT

Not applicable.

7. NON-INVESTIGATIONAL PRODUCT

Not applicable.

8. METHODS

○ Study parameters/endpoints

▪ Main study endpoints

- Establishment of an *in vitro* assay to study recognition and killing of *P. falciparum*-infected hepatocytes by:
  - CSP-specific cytolytic CD8+ T cells
  - hepatic and peripheral innate/innate-like lymphocytes

▪ Secondary study endpoints

- Recognition (IFNγ and CD107a expression) and killing (lysis or apoptosis) of *P. falciparum*-infected hepatocytes by cytolytic CD8+ T cell line
- Differences in recognition and killing of *P. falciparum*-infected hepatocytes between liver-resident and peripheral blood lymphocytes
- Identity of the individual lymphocyte (sub-)populations which contribute to recognition and killing of *P. falciparum*-infected hepatocytes

▪ Exploratory study endpoints

- Optimal time point during intra-hepatocytic development (early, middle or late) for recognition and killing of *P. falciparum*-infected hepatocytes
- Difference in recognition and killing of *P. falciparum*-infected hepatocytes between parasite strains
- Comparison of recognition and killing of *P. falciparum*-infected hepatocytes in different zonal hepatocyte types
- Characterisation of immunological pathways involved in recognition and killing of *P. falciparum*-infected hepatocytes by lymphocyte (sub-)populations

○ Randomisation, blinding and treatment allocation

This is a non-randomised, open-label study, in which all participants will undergo the same study procedures (blood collection).

○ Study procedures

▪ Subject inclusion

Participants will be recruited amongst patients presenting to Radboudumc Department of Surgery for medically-indicated partial liver resection for underlying disease. Patients are first

seen by their practitioner (surgeon) as part of their treatment program (intake day), without the research nurse being present. The practitioner will make a pre-selection based on the already known Inclusion and Exclusion Criteria. Only in patients who seem to meet these criteria, and only if the patient gives the practitioner permission to do so, will the trial nurse be instructed by the practitioner to subsequently approach potential participants (i.e. on the same day, but in a different room, without the practitioner being present) with information about the study. The trial nurse will provide potentially interested subjects with the information letter and informed consent forms. After the potential subject has had at least 1 day to consider, the trial nurse will call him/her to answer any remaining questions and, if the subject agrees, ask them to sign the Informed Consent and return it by mail or bring it with them to their next scheduled visit in the context of their treatment program (usually their anaesthesia consultation).

- **Blood sampling**

6mL of peripheral venous whole blood will be obtained as soon as possible after obtaining informed consent, usually during the patient's anaesthesia consultation, for determination of HLA-A2 phenotype. This blood draw should take place at least 7 days prior to scheduled surgery in order to allow sufficient time to expand the CD8 T cell line to coincide with the availability of freshly-isolated hepatocytes in HLA-A2+ donors.
A further 24mL of whole blood will be drawn immediately prior to liver surgery through an existing intravenous or arterial line. (If in an individual subject it is impossible for logistical reasons to obtain the first 6mL blood sample sufficient early relative to their scheduled operation, then only the pre-operative 24mL sample will be collected and this will be used as for HLA-A2-negative donors.)

- **Partial liver resection**

Subjects will undergo medically-indicated routine partial liver resection for their underlying disease. Due to the anatomy of the liver and the location of the tumour, often a large rim of healthy liver tissue is removed in order to obtain these clear resection margins. Any such resected liver tissue that is not required for diagnostic purposes in the context of the patient's underlying disease (and would thus otherwise be considered medical waste), may be used for isolation of healthy hepatocytes liver-resident immune cells for study purposes. Although it cannot be excluded that this macroscopically health tissue contains microscopic tumour deposits, generalised interstitial liver disease or cirrhosis would not be expected in this category of patients undergoing surgery and subjects with hepatitis B or C will not be

included. Despite these limitations, the use of fresh primary hepatocytes obtained from such liver surgeries is considered superior to the use of currently available hepatocyte cell lines.

- **Other clinical procedures**

Patients will receive routine clinical care for their underlying disease, which falls outside of the scope of this study.

- **Laboratory procedures**

- **Isolation of PBMCs**

PBMCs can be isolated by centrifugation with Ficoll gradients and individual subpopulations further purified through the use of Percoll gradient centrifugation, fluorescence activated cell sorting (FACS), or magnet-activated cell sorting (MACS). A portion of the isolated cells will be frozen in liquid nitrogen for optional later analyses.

- **HLA-A2 phenotype determination**

Phenotypic HLA-A2 expression will be measured by flow cytometry on participants' PBMCs by flow cytometry. This will be performed at Inclusion, i.e. several weeks before scheduled surgery, in order to allow time to expand the HLA-A2-restricted CSP-specific CD8+ T cell line to coincide with the availability of fresh human hepatocytes from partial liver resection in HLA-A2+ patients.

- **Isolation of hepatocytes and intrahepatic immune cells**

Hepatocyte isolation and culture is an established procedure in our laboratory (11). Briefly, individual hepatocytes are obtained by perfusing the liver explant with collagenases followed by manual preparation of the liver into small fragments and manual digestion into cell medium. Cell suspensions are passed over a 100µM cell strainer and the subsequent preparation is centrifuged repeatedly at 8 x*g*. The resultant cell pellet consists primarily of hepatocytes, which are cultured in 96-well tissue culture plates. The supernatant can be further purified using 35% Percoll continuous gradient centrifugation to obtain intrahepatic immune cell populations. Further purification by FACS or MACS can be performed to isolate specific innate cell sub-populations like NK cells or γδ T cells. If the subject provides consent for storage of samples, excess hepatocytes may be cryopreserved and stored in liquid nitrogen to be thawed at a later date for short-term use in (a) separate experiment(s).

- **Expansion of CSP-specific CD8+ T cell line**

Expansion of the HLA-2A-restricted CSP-specific CD8+ T cell line will be performed according to established laboratory procedures. Since this process takes 2-3 weeks, it must be initiated in advance of scheduled surgery in patients shown at Inclusion to express HLA-2A, to coincide with the availability of HLA-matched fresh human hepatocytes from partial liver resection in these patients.

- **Analysis of antimalarial immune responses to liver-stage *P. falciparum***

Cultured hepatocytes will be infected with *P. falciparum* sporozoite-stage parasites as per established protocols (18). Briefly, sporozoites dissected from the salivary glands of infected mosquitoes will be added to cultured hepatocyte plates 48h post-seeding and centrifuged at 300x*g* to ensure cell-to-cell contact.

Recognition of *P. falciparum*-infected hepatocytes by immune cells (CSP-specific $CD8^+$ T cells, liver-resident or peripheral immune cells) will be determined primarily by the following parameters:

- Cytokine production (% IFNγ + lymphocytes)
- Degranulation (% CD107a + and / or granzyme B + lymphocytes)
- Proliferation (% Ki67 + lymphocytes)
- Memory induction (% CD25 + CD45RO + CD62L + lymphocytes)

The above parameters will be measured using flow cytometry on immune cells cultured with *P. falciparum*-infected or (control) uninfected hepatocytes after (intracellular) staining with fluorescent monoclonal antibodies.
Killing of *P.f.*-infected hepatocytes will be measured by counting the number of (fluorescent) intact intracellular parasites per well, in the presence and absence of CSP-specific CD8 + T cells, liver-resident or peripheral immune cells, by means of. fluorescence microscopy. Experiments using expanded CSP-specific CD8+ T cells will be performed in hepatocyte cultures from all participants shown at Inclusion to express HLA-A2. Recognition and killing by liver-resident and peripheral innate(-like) lymphocytes will be assessed in all subjects with sufficient material, regardless of HLA-A2 phenotype or if this remains unknown (e.g. if it was impossible to obtain the 6mL sample for phenotyping in sufficient time).

- **Withdrawal of individual subjects**

Subjects can leave the study at any time for any reason if they wish to do so, without any consequences. Any of the subject's stored samples will then be destroyed. The investigator can decide to withdraw participants from the study for urgent medical reasons.

SAFETY REPORTING

- **Temporary halt for reasons of subject safety**

Since the risks associated with study-related procedures (blood-collection) are minor, it is not anticipated that the study will need to be temporarily halted, either at an individual or study level.

- **AEs, SAEs and SUSARs**

Non-serious AEs associated with study-related blood-collection will not be reported. It is considered extremely unlikely that any study-related SAE will occur, but if it does it will be reported to CMO following Dutch national (WMO/CCMO) guidelines.

9. STATISTICAL ANALYSIS

- **Primary study parameter(s)**

Recognition and killing of *P. falciparum*-infected hepatocytes by the CSP-specific cytolytic CD8+ T cell line and liver-resident or peripheral immune cells, will be assessed in paired-samples *t*-tests or nonparametric equivalents for single variables. Repeated measures ANOVA with appropriate adjustment for multiple comparisons will also be used to test significance between multiple variables.

- **Secondary study parameter(s)**

Comparisons between liver-resident and peripheral immune cell populations and between different lymphocyte (sub-)sets will be assessed using paired-samples *t*-tests or nonparametric equivalents and ANOVA with appropriate adjustment for multiple comparisons, respectively.

- **Other study parameter(s)**

Exploratory endpoints will be assessed descriptively or using applicable statistical tests if sufficient data is available.

- **Interim analysis (if applicable)**

Not applicable.

10. ETHICAL CONSIDERATIONS

- **Regulation statement**

This study will be conducted in accordance with the latest version of the Declaration of Helsinki and the Medical Research Involving Human Subjects Act (WMO).

- **Recruitment and consent**

Patients will first be seen by their treating physician upon initial scheduling for surgery (usually during an outpatient visit), who will perform a pre-selection based on known In- and Exclusion criteria. Potentially suitable subjects who give their practitioner permission for this will be invited to next meet with the trial nurse, who will inform them about the study and provide them with the information letter and informed consent forms. After the potential subject has had at least 1 day to consider, the trial nurse will call him/her to answer any remaining questions and, if the subject agrees, ask them to sign the Informed Consent and return it by mail or bring it with them to their next scheduled visit in the context of their treatment program (usually their anaesthesia consultation). Upon obtaining informed consent, it is necessary to perform the first (6mL) blood draw for HLA-A2 phenotyping as soon as possible, in order to allow sufficient time (minimum 7 days) for expansion of the cytolytic CD8+ T cell line prior to scheduled surgery to coincide with the availability of fresh liver tissue for culture.

- **Benefits and risks assessment, group relatedness**

There is no direct benefit to study participants. Malaria poses a significant risk to global health and a vaccine is urgently needed to combat the burden of disease. Development of a vaccine against the liver stage would prevent malaria-related morbidity and mortality entirely. Unfortunately, very little is known about liver-stage immunity. An *in vitro* liver stage platform to investigate immunity to *P. falciparum* in the liver would advance the field significantly by enabling more in-depth studies of the correlates of protection and factors which can modify the host immune response.

In the proposed study, adult patients scheduled for medically-indicated partial liver resection for underlying disease will undergo one 6mL blood draw to determine HLA-A2 phenotype and another 24mL blood draw on the day of surgery, through an existing intravenous or arterial line. The risks associated therewith are considered minimal. The liver tissue obtained for this study would otherwise be discarded as medical waste and thus represent no additional risk to participants.

- **Compensation for injury**

The Arnhem-Nijmegen Region Human-related Research Committee has granted exemption from the obligation to take out insurance for this research. The Committee is of the opinion that this study by its nature is without risk for the participants.

- **Incentives (if applicable)**

None applicable.

11. ADMINISTRATIVE ASPECTS, MONITORING AND PUBLICATION

o Handling and storage of data and documents

All parties agree to adhere to the principles of medical confidentiality in relation to patients included in this study, and shall not disclose the identity of patients to third parties without prior written consent of the subject.

Information with regards to a patient's age, gender, underlying and associated disease, and surgical procedure will be collected. This information will be collected in a database labelled only with a pseudonymous study code. All biological samples will be labelled with a study code only. On condition the subject provides consent, samples will be stored for 15 years in the coded form (study name and patient study code).

Identifying information (full name and date of birth) will be collected only as necessary to obtain informed consent. Informed consent forms and patient identification list will be kept in a secure location by the trial nurse, who forms part of the surgical team but is not directly involved in the (immunological) research.

Stored biological samples can be used by the researchers for the objectives as described in section 2. For certain objectives samples may be analysed at sites outside the Radboudumc. These samples will be labelled only with the study code. Parties outside the Radboudumc will not have access to identifying information. Permission will be asked from the METC prior to use of samples for any objectives not listed in section 2 or if there is a chance of incidental findings that may be clinically relevant to individual patients. If a subject withdraws his/her consent at any time, their respective pseudonymised samples will be destroyed.

o Monitoring and Quality Assurance

As this is an observational study with minimal risk to participants, no investigatory product and with a single 6mL venepuncture and an additional 24mL blood draw from an existing lines as the sole interventions, exemption from monitoring requirement is requested.

o Amendments

Amendments are changes made to the research after a favourable opinion by the accredited METC has been given. All amendments will be submitted for approval to the METC that gave the initial favourable opinion.

o Annual progress report

The sponsor/investigator will submit a summary of the progress of the trial to the accredited METC once a year. Information will be provided on the date of inclusion of the

first subject, numbers of subjects included and numbers of subjects that have completed the trial, serious adverse events/ serious adverse reactions, other problems, and amendments.

- **Temporary halt and (prematurely) end of study report**

The investigator/sponsor will notify the accredited METC of the end of the study within a period of 8 weeks. The end of the study is defined as the inclusion date of the last patient.

- **Public disclosure and publication policy**

A final report will be prepared by the investigators at the Radboudumc and will be signed by the investigator. The investigator and sponsor will make every effort to publish the results in a peer-reviewed journal.

12. STRUCTURED RISK ANALYSIS

Not applicable, as there is no investigatory product.

14. REFERENCES

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