

Biological sampling / biobanking summary for TIGERS trial – DRAFT

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Background

The collection of biological samples within the TIGERS trial presents a unique opportunity to add additional value to the study. It will allow us to explore mechanistic questions (such as the biology underpinning differential response to therapies across subphenotypes), discovery and evaluation of additional phenotyping approaches and the generation of an extensive biobank composed of multiple samples per patient spanning diverse sample types and timings. The unique combination of multi-specimen longitudinal sampling coupled to prospective gene-expression subphenotyping, randomized drug interventions and detailed clinical data provides an unmissable opportunity to address these types of question. We anticipate that biobank generated in this trial will also help to build future translational research capacity by providing samples and data to support high quality PhD proposals as well as early and mid-career academics research projects and international collaborations. Within our group we have extensive experience of such an approach its ability to deliver impactful research¹⁻⁶. This sampling plan outlines the collection of samples for DNA extraction, transcriptomic analysis, and cytokine measurement in the TIGERS trial. The plan is designed to initially support both mechanistic studies and optimization of phenotyping approaches without impacting recruitment to the main trial. Funding within the TIGERS trial will support collection of baseline samples, further funding will be sought for longitudinal and additional sampling. It is anticipated that additional sample types will be added during the lifespan of the trial based on emerging evidence in the field.

Consent for research sample collection

Consent for research sample collection and their use in future research projects will be embedded into the process for consent for TIGERS to minimise burden on patients. We will look at consent procedures with the PPIE lead and patients and the public to ensure that relatives aren't overburdened.

Sampling schedule

Blood samples will be collected at three timepoints: Day 0 (prior to randomization), Day 3, and Day 7, using a tiered approach at site level depending on facilities and resources. The following sample types will be obtained:

- Lithium Heparin (LiHep) Tubes: For plasma cytokine analysis including IL-6 and TNF- α .
- EDTA Tubes: For plasma collection and downstream biomarker analysis.

- Serum Separating Tubes (SST): For antibody and biomarker analyses.
- PAXgene RNA Tubes: For transcriptomic profiling and RNA extraction.
- CPT citrate Tubes: For isolation of peripheral blood mononuclear cells (PBMCs) for DNA extraction.

| Tier | Time point | Stratification sample | LiHep | EDTA | SS A | PAXgene RNA | CPT |
|------|-----------------------------|-----------------------|-------|------|------|-------------|-----|
| 0 | Baseline | x | | | | | |
| 1* | Baseline | x | x | x | x | x | |
| 2 | Baseline/Day 3/Day 7 | x | x | x | x | x | |
| 3 | Baseline/Day 3/Day 7 | x | x | x | x | x | x |
| 4 | Baseline/Day 3/Day 7/Day 90 | x | x | x | x | x | x |

Samples will be processed according to standardized protocols and stored at site until batch shipment to the central laboratory. All samples will be labelled with unique identifiers and tracked using electronic case report forms (eCRFs).

Downstream analysis

Sample collection guidelines will be developed to ensure that collected samples are widely suitable for downstream analytical processes. Specific analytical procedures will be determined to best address mechanistic questions that arise during the lifetime of the TIGERS trial.

References

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6. Antcliffe DB, Burnham KL, Al-Beidh F, et al. Transcriptomic Signatures in Sepsis and a Differential Response to Steroids. From the VANISH Randomized Trial. *Am J Respir Crit Care Med* 2019;199(8):980–6.