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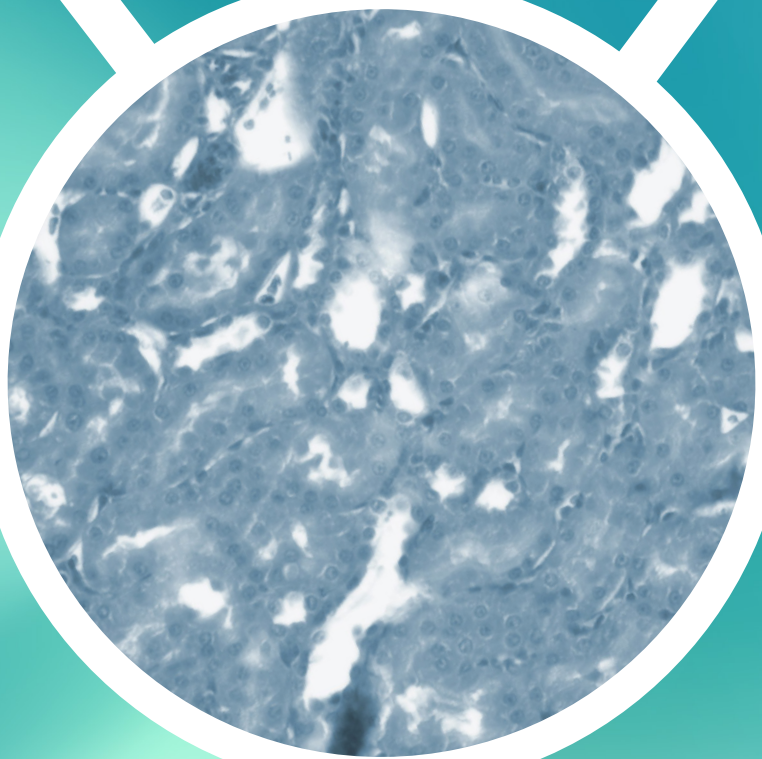
discovery services

Successfully conducting Tissue Cross-Reactivity studies



Introduction

Developing a successful novel therapeutic is incredibly challenging and requires significant investment of both time and capital. According to Deloitte's 15th Annual Pharmaceutical Innovation Report (2025) the average cost of bringing a single new therapeutic to market was nearly $2.2 billion, including expenditures on failed trials[1]. Given this, it is important to identify as early as possible during development any potential issues with a new therapeutic—both to limit failures in late-stage studies and to minimise the potential for harm in clinical trials. Many of the preclinical investigations that constitute essential components of an Investigational New Drug (IND) application or Clinical Trial Application (CTA) are intended to minimise the risk of harm in first-in-human studies. For therapeutic antibodies or antibody-like molecules, preclinical Tissue Cross-Reactivity (TCR) studies have become a key tool for gathering and assessing important data regarding on- and off-target binding.

In this white paper, we explore the role and value of TCR studies in therapeutic development. We also discuss critical considerations for developing a rigorous study that generates the robust data necessary to support preclinical decision-making and regulatory submissions.

Background on TCR studies

TCR studies are recommended for antibody and antibody-like molecules that contain a complementarity-determining region (CDR). These studies consist of a series of immunohistochemical (IHC) screening assays that are conducted not only to identify off-target binding, but also to detect previously unknown sites of on-target binding for a novel biotherapeutic.

The presence of off-target therapeutic antibody binding in frozen *ex vivo* tissues is used to provide insight into potential organ toxicity *in vivo*. Identification of new sites of on-target binding offers the possibility of expanding the potential indications for the biotherapeutic.

Studies that compare target expression patterns between human and animal tissue can be used to rationalise organ-specific toxicities found in the preclinical species and predict how those findings might translate into potential safety issues in humans. These data may include *in vivo* toxicity studies and *in vivo* pharmacodynamic modelling studies. TCR evaluation of animal tissues may also be useful for providing supplemental information regarding potential correlations, or lack thereof, with preclinical toxicity when there is unexpected binding in human tissues[2].

Although there is much debate on whether staining in TCR studies correlates with organ toxicity in a clinical environment, regulators do require these studies in the preclinical safety assessment package for IND/CTA submissions for most biotherapeutics.

TCR study development

One of the most important aspects in designing a TCR study is the development and optimisation of the IHC protocol to be used. Novel biotherapeutics are designed as drug molecules and are not inherently optimised to be ideal IHC assay development tools or reagents. This can therefore pose a technical challenge that requires a rigorous assay workup and, potentially, multiple rounds of assay development. Additionally, and crucially, a favourable dataset for a TCR study is a broadly negative result—representing no off-target binding—and therefore it is critical to validate that the assay is specific and robust before examining test tissues in order to rule out any false negative results in the TCR study.

Researchers often underestimate the length of time needed to develop a scientifically acceptable IHC method, leading to study delays. Working with a TCR assay service provider who has deep expertise in IHC assay development and optimisation can help to streamline the process and help ensure studies stay on schedule.

Considerations for study design

TCR studies may be conducted in compliance with Good Laboratory Practice (GLP) or under non-GLP conditions. Researchers may opt to perform non-GLP tissue microarray (TMA) screening for initial assessments of biotherapeutic candidates. For example, TMA screening can be used to prioritise or eliminate candidates based on off-target binding. For regulatory submissions, TCR studies must be conducted under GLP according to the published guidance, which recommends use of GLP or GMP (Good Manufacturing Practice) grade candidate molecule, referred to as the Test Item. The guidance calls for evaluation of the Test Item at 2 concentrations in 3 different, unrelated human donors and appropriate preclinical species. In practice, however, the majority of studies conducted evaluate a GLP-grade Test Item at a single, optimised concentration and focus primarily on generation of data in the required human tissue cohort. Where other animal species are included in these studies, the experimental approach is the same; however, these studies typically do not claim GLP compliance as they are considered to be supplementary research data.

Considerations for Test Items

Test Items—the biotherapeutics to be administered in first-in-human studies—come in a variety of forms, some of which differ substantially from immunoglobulins in structure. Regardless of the format of the Test Item, consideration must be given to how the molecule will be detected in an IHC assay. Unlabeled human or humanised antibodies can be detected by pre-complexing with an anti-human antibody before application to test tissues. From a technical perspective, however, it is easier to work with an antibody that has been labeled with a small molecule, such as biotin or a fluorescent dye such as fluorescein isothiocyanate (FITC) or one of the Alexa fluorophores, that can be detected with a label-specific antibody. For many molecules, such labels are necessary for facilitating detection.

Biotinylation is a well-established and relatively straightforward technique for labeling biological molecules, but it requires additional avidin-biotin blocking steps in the assay protocol to avoid issues with background staining due to endogenous biotin in human tissues. Consequently, fluorescent labels are preferred in a TCR study. With either label, it is important to establish the impact of labeling on the binding properties of the molecule and to ensure Test and Control Items are labeled to the same degree.

Considerations for Control Items

Inclusion of a Control Item in a TCR study is strongly recommended[3]. The Control Item is typically a species- and isotype-matched non-immune IgG if the Test Item is an antibody, or a molecule that is identical in structure to the Test Item but binds a molecule that is unlikely to be found in human tissue—for instance, green fluorescent protein, a plant protein, or snake venom. Ideally, the Control Item is prepared in parallel with the Test Item, including any necessary labeling, and is used to ascertain the background level and pattern of tissue binding that occurs irrespective of the CDR.

Failing to include a suitable Control Item for comparison may result in interpretation of any binding of the Test Item as specific, which can be misleading. While there are other methods for assessing binding specificity, such as preincubating with a molar excess of soluble antigen to compete for binding, these methods generally add to overall study cost and may not be feasible due to limited availability of soluble antigen.

Considerations for positive control material

Selecting a suitable positive control material is also crucial for TCR protocol development and GLP studies. Positive control material is used in the IHC assay development and to validate the Test Item binding in all of the assay runs, and ideally is a frozen tissue sample.

Frozen tissue is superior to overexpressing cell lines or other types of positive control material because it retains tissue matrix. If there are no suitable tissues that naturally express the target of interest, alternative techniques can be employed; for example, incorporation of soluble antigen into a human tissue matrix (see Table 1).

Considerations for test tissues

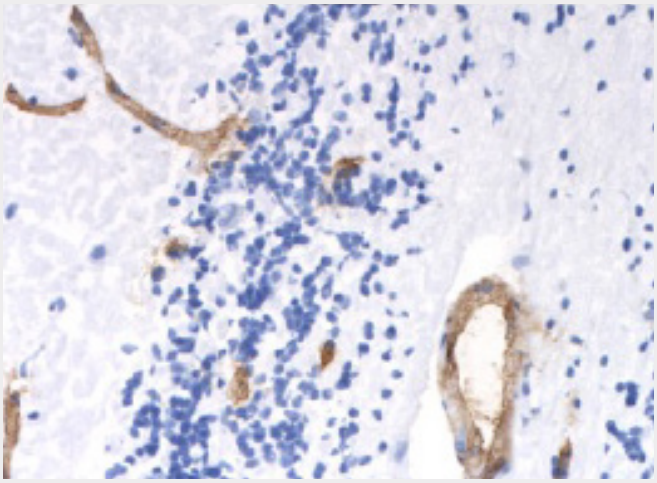
FDA and EMA guidelines for the development of therapeutic antibodies and related products recommend TCR testing on a range of human tissues[4,5]. According to the FDA's Points to Consider in the Manufacture and Testing of Monoclonal Antibody

Table 1: Alternative techniques for IHC assay development

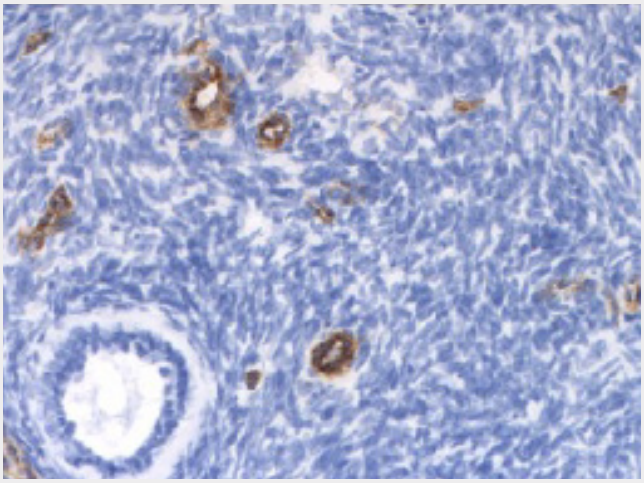
Scenario	Case example
Non-human targets	When developing assays for a non-human target, such as SARS-CoV-2, where the use of frozen, infected tissues is not an option due to either availability or safety considerations, cell lines expressing the protein or epitope to which the antibody is targeted are an effective alternative. Positive and negative expressing cell lines can be included in any aspect of IHC assay development. Assay conditions may need to be modified, however, when transferring to frozen tissues due to the propensity for higher non-specific binding, which can be reduced through standard approaches such as protein/serum or peroxidase blocking.
Modified proteins	When developing methods to detect a non-naturally occurring protein, homogenised tissue samples spiked with recombinant target protein can be created.
Antibody-drug conjugates	Anti-linker antibodies have been successfully used to specifically detect Test Item binding in TCR assays for antibody-drug conjugates.
Test Items that cannot be labeled	When a Test Item cannot be labeled, it is not possible to use an anti-label secondary antibody. An alternative approach is to pre-complex the Test Item with anti-human IgG secondary antibody before application to the tissues.
Multi-specific binding molecules	For bi-specific molecules, there is no regulatory requirement to study the individual binding components, just the bi-specific molecule itself. However, it is not then possible to determine if any observed binding of the bi-specific is due to binding to just one or both of the targets. For one such molecule, we were asked to provide additional context and data. Thus, we generated supplementary non-GLP TCR data with commercial antibodies to each of the individual binding components to demonstrate target-specific patterns of staining.

Products for Human Use, frozen tissue is recommended[4]. In GLP TCR studies, the quality of the frozen tissues used is of the utmost importance. These tissues must retain antigenicity and exhibit good morphological preservation to allow adequate interpretation of staining patterns. Incorporating confirmation of tissue antigenicity into the GLP study is strongly recommended, as testing prior to the GLP study does not guarantee antigenicity once the tissue has been sectioned onto glass slides. A confirmatory assay commonly used to validate the antigenicity of tissues used in TCR studies involves immunostaining of tissues with von Willebrand Factor (vWF) antibodies (see Figure 1).

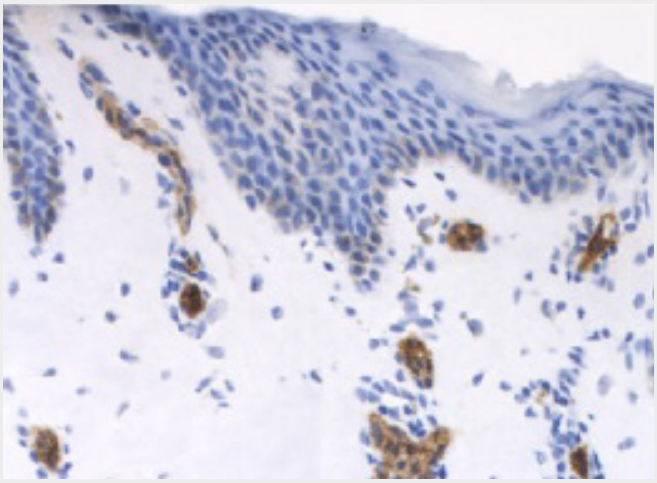
Figure 1: Confirming tissue antigenicity with vWF immunostaining



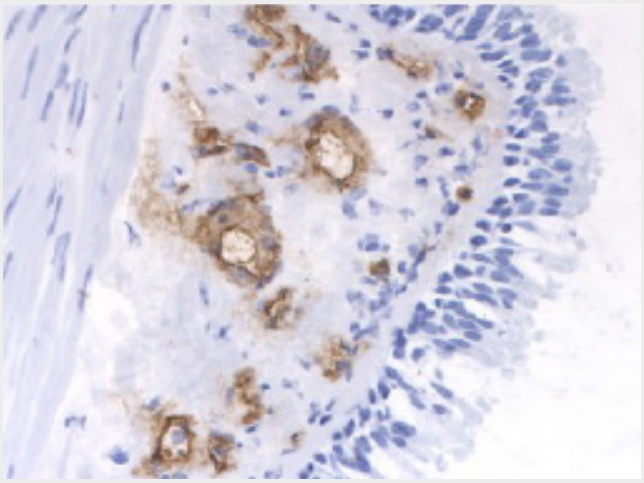
Cerebellum



Ovary



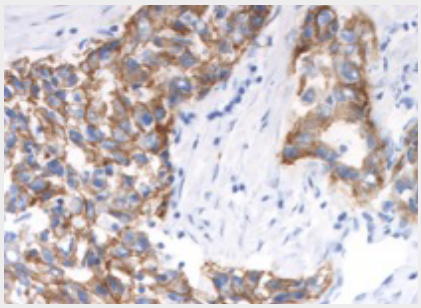
Skin



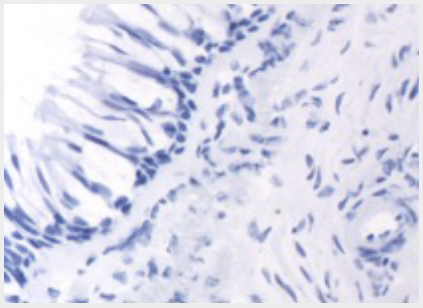
Bronchus

Images show the binding of vWF antibodies (brown staining) to the vascular endothelium in frozen sections of cerebellum, ovary, skin, and bronchus.

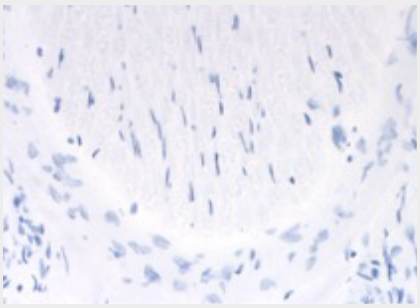
Figure 2: Example of GLP TCR data



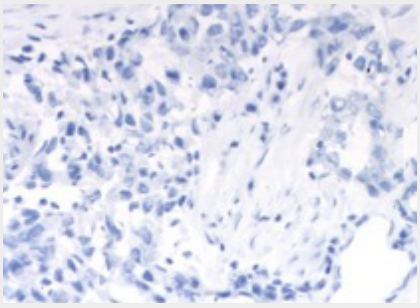
Test item in positive control tissue



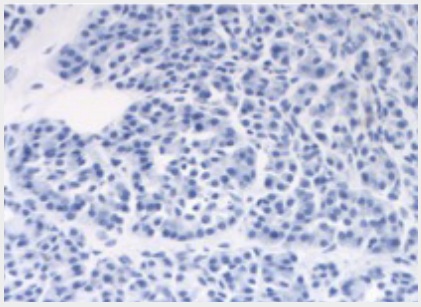
Bronchus



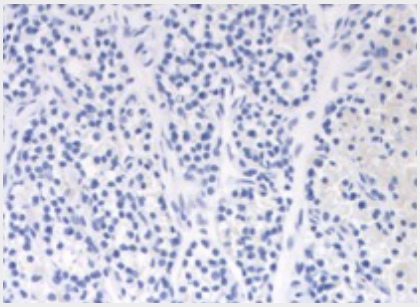
Peripheral nerve



Control item in positive control tissue



Pancreas



Parathyroid

Left-hand panels show the optimised IHC assay binding of Test Item and Control Item to the positive control tissue.
Right-hand panels show the absence of Test Item binding to a variety of frozen tissues.

Interpreting TCR study results

TCR study results should be interpreted by a qualified pathologist. Staining observed with the Test Item should be compared to that seen in adjacent sections that have been incubated with the Control Item (see Figure 2). Specific staining should be considered only where Control Item staining is absent or if the Test Item staining is clearly more intense than the Control Item staining. If staining is smeared or very diffuse, the pathologist will determine whether that staining is specific.

The cellular location of specific staining should also be factored into the interpretation of TCR study results. Staining of cytoplasm, for example, is less likely to translate into a biological effect or safety concern than membrane staining because cytoplasm is unlikely to be accessible to a biotherapeutic *in vivo*. Therefore, binding should be evaluated and interpreted based on the overall pharmacology and safety assessment data package.

The biological relevance of any TCR staining can only be validated when other human safety or toxicity data, such as clinical trial or post-marketing surveillance data, become available. In part, this is because TCR studies are performed on pathologically normal tissue, while patients treated with the biotherapeutic have a disease condition. The target expression profile may be differentially expressed in both magnitude and distribution in the normal and diseased states. Therefore, TCR study data must be interpreted carefully in the context of preclinical studies on a case-by-case basis to inform development decisions.

Choosing a TCR provider

Pharmagene Discovery Services offers a unique combination of scientific expertise, high-quality human tissues and a track record of developing binding assays for a range of biotherapeutic modalities to support the conduct of non-GLP and GLP TCR studies. As an expert in IHC assay development, we have developed assays for monoclonal antibodies; mono-, bi-, and tri-specific antibody-like molecules; scFv fragments; and antibody-drug conjugates.

Our proven 2- or 3-phase approach, outlined below, provides a cost-effective solution for making confident decisions regarding the best parameters for a study, minimising the risk of GLP study failure. The output of our GLP TCR service is a report that is suitable for submission as part of an IND or CTA.

Availability of qualified specimens

Pharmagene has developed, and maintains, a well-curated, dedicated panel of high-quality frozen human tissues to deliver TCR studies. All human tissues recommended for TCR testing by the FDA and the EMA are fully consented for commercial research, and available from at least 3 male and 3 female donors (see Table 2).

Table 2: Tissue types available for TCR testing

Tissue types are available from multiple male and female donors		
Adrenal gland	Ileum	Prostate
Bladder	Kidney – glomerulus and tubule	Skeletal muscle
Blood cells	Liver	Skin
Blood vessel endothelium	Lung – bronchus and parenchyma	Spinal cord
Bone marrow	Lymph node	Spleen
Breast	Ovary	Stomach
Cerebellum	Pancreas	Testis
Cerebral cortex	Parathyroid gland	Thymus
Colon	Parotid salivary gland	Thyroid gland
Eye	Peripheral nerve	Tonsil
Fallopian tube	Pituitary gland	Ureter
Heart	Placenta	Uterus – cervix and endometrium

All specimens undergo a 4-point inspection to qualify for GLP TCR studies:

1. Evaluation of donor clinical history to ensure experimental suitability
2. Review by board certified pathologists to validate normal morphology/pathology
3. Confirmation of compliance with ethical, legal, and regulatory requirements
4. Initial confirmation of tissue antigenicity

Pharmagene has also built a set of proprietary frozen TMA that can be used for non-GLP TCR studies which provide an economical and rapid turnaround approach for screening candidate molecules. Our panel of 3 TMAs contains the 36 tissues required by the FDA and EMA, accelerating the de-selection of candidates that exhibit significant off-target immunoreactive profiles.

A flexible approach

Pharmagene utilises a 2-3-phase approach to TCR studies to help ensure quality data (see Figure 3)

Phase 1 – Assay Optimisation.

The Test Item and Control Item are incubated on both positive and negative control tissues at multiple concentrations and different tissue fixation conditions to determine the optimal specificity and staining conditions for immunohistochemical detection. The output of this phase is a report outlining the data and recommended final methodology.

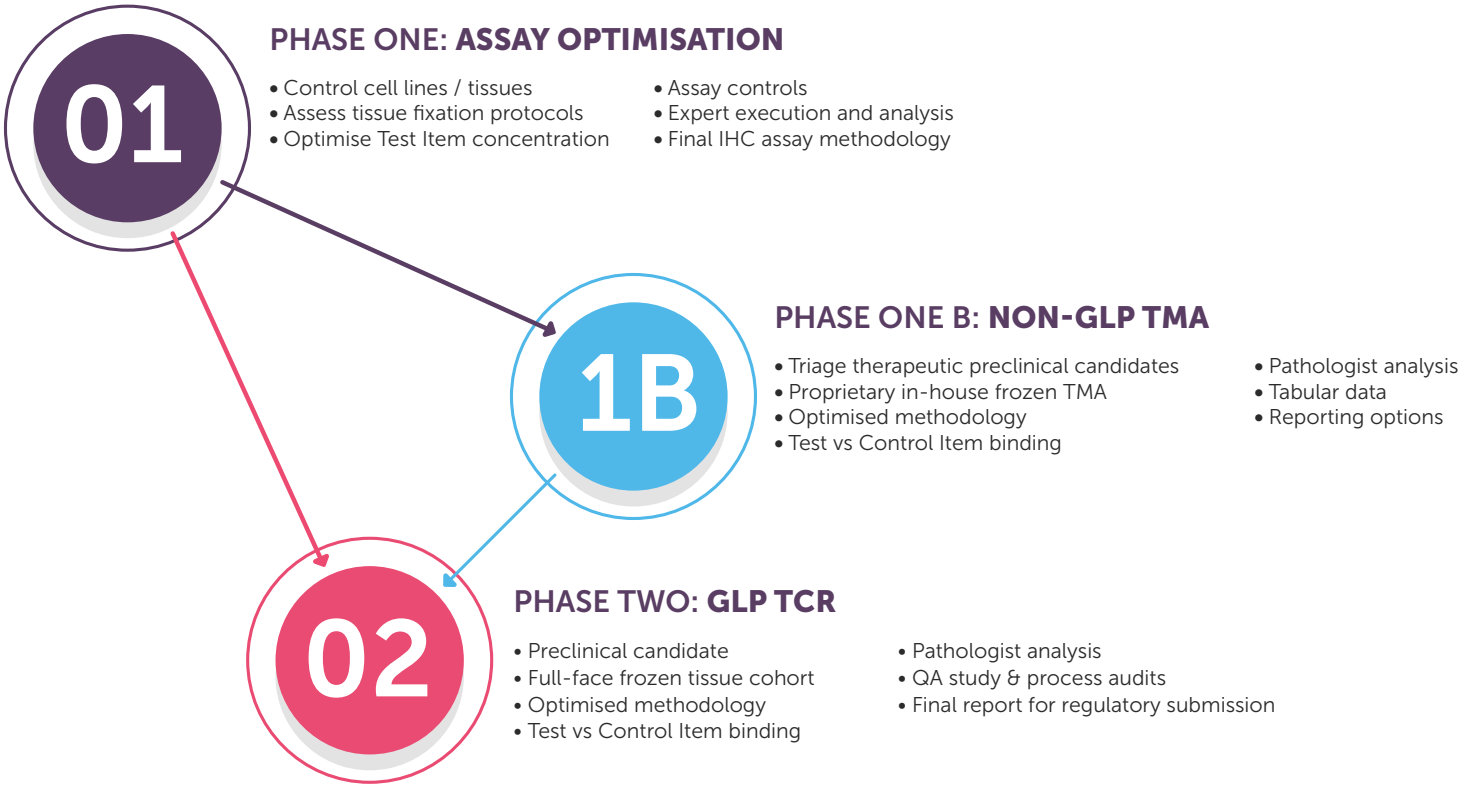
Phase 1b – TMA Screening (non-GLP).

The optimised assay protocol is performed on proprietary frozen TMA sections, allowing efficient, cost-effective triage of multiple therapeutic candidates or earlier evaluation of molecules during lead optimisation. The Phase 1b report includes methodology, tissue details, and pathologist generated data on the presence or absence of Test Item binding.

Phase 2 – GLP TCR (see Figure 2).

This assay uses full-face, frozen tissues and results undergo review by a qualified pathologist. The final GLP Study Report generated from this study is suitable for regulatory submission.

Figure 3: Our 3-Phase approach provides quality data for your study



Conclusion

TCR studies are an important aspect of the preclinical development of therapeutic antibodies and antibody-like molecules as off-target binding can result in treatment-related toxicity. Performing optimised TCR assays on well-characterised, high-quality specimens under GLP conditions is essential for generating the robust data needed to manage development risk and support regulatory submissions. Working with a solutions provider that combines biospecimen availability with expertise in IHC assay development can help accelerate the development of a therapeutic antibody candidate programme.

About Pharmagene Discovery Services

Originally founded in 1996 and operating from laboratories near Cambridge, UK, Pharmagene Discovery Services is built upon the scientific knowledge, expertise and reputation of its legacy, and remains one of the earliest established companies to focus on human tissue-based research. Leveraging these skills and experience, it specialises in tailored assay design and development, with range of research services encompassing target expression, interrogation of spatial biology, functional cellular biology and bioassays, and biotherapeutic safety testing human tissues and primary human cells. The facility operates to GLP and is licenced by the UK Human Tissue Authority.

Pharmagene has created and curates an in-house human tissue biorepository to support research for its clients, and in addition offers sample processing and biostorage services. With a wealth of knowledge of the human tissue research sector, and having conducted hundreds of studies for life science researchers around the world over the past three decades, the business is uniquely placed to support its clients to develop safe and efficacious new therapies and associated biomarkers more rapidly and with greater confidence.

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