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MINI-REVIEW

Best practices for blood collection and anaesthesia in mice: Selection, application and reporting

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Abstract

Blood collection in mice is a common procedure in biomedical research. The choice of blood collection method and the need for analgesia and/or anaesthesia depend on multiple factors, including the experimental setup, animal welfare considerations and the intended downstream analyses. This minireview describes key non-surgical and surgical blood collection techniques, the appropriate use of analgesia and anaesthesia, and the best practice for documentation and adherence to reporting standards in animal studies. We here provide a table summarising collection procedures; a table listing animal welfare guidelines from multiple countries; a table describing the most common analgesics and anaesthetics, with doses and route of administration; and a table outlining key points for reporting blood collection, anaesthesia and analgesia protocols. A decision chart is also included to assist in selecting the most suitable method. Ultimately, with this minireview, we aim to promote standardised practices, improve data reproducibility, and support ethical animal research.

KEYWORDS

3R principles, anaesthetic protocols for mice, animal experiment ethics, animal experiment reporting, animal research regulations, animal welfare guidelines, bleeding methods comparison, blood collection site in mice, mice anaesthesia and analgesia, mice blood collection, microsampling, mouse blood collection and welfare, refinement of blood sampling in mice, reporting standards for mouse blood collection, retro-orbital blood collection

1 | INTRODUCTION

Blood collection in mice is a common procedure in biomedical research. A key ethical challenge in animal research is balancing scientific advancement with minimising pain and distress to the animals involved. The *3R principles—Replacement, Reduction, and Refinement*—were introduced in 1959 by William M.S. Russell and Rex L. Burch in their seminal work *The Principles of Humane Experimental*

Abbreviations: ASPA, Animals Scientific Procedures Act; FELASA, Federation of European Laboratory Animal Science Associations; MOST, Ministry of Science and Technology; NC3Rs, National Centre for the Replacement, Refinement and Reduction of Animals in Research; NIH, National Institutes of Health; OACU, Office of Animal Care and Use; 3R principles, Replacement, Reduction, and Refinement.

For affiliations refer to page 2349

Technique (Russell & Burch, 1959). They defined *humane research* as any scientific investigation conducted with respect for the well-being of all living subjects, prioritising the minimisation of pain and distress. To implement humane research and ensure ethical standards, the 3R principles advocate for: *Replacement* (using alternatives to animals when possible), *Reduction* (using the minimum number of animals required for valid results) and *Refinement* (improving experimental procedures to minimise suffering) (Hubrecht & Carter, 2019; Richter, 2024; Russell & Burch, 1959). These principles have now been embedded in national and international legislation and regulations on animal research, as well as in the policies of organisations that fund or conduct animal research (Animals Scientific Procedures Act, 2017; Federation of European Laboratory Animal Science Associations [FELASA]: FELASA Working Group, 2023). By applying the 3R principles, experiments involving animals can produce scientifically valid results while maintaining ethical responsibility (Hubrecht & Carter, 2019; Richter, 2024).

This mini-review discusses key blood collection techniques, the appropriate use of analgesia and anaesthesia, and the best practices for documentation and adherence to reporting standards in animal studies, in accordance with the 3R principles, Good Laboratory Practice and Good Scientific Practice. Specifically, we here provide a table summarising collection procedures; a table listing animal welfare guidelines; a table describing most common analgesics and anaesthetics, doses and route of administration; and a table outlining key points for reporting blood collection, anaesthesia and analgesia protocols. A decision chart is also included to aid in the decision-making process. The impact of different blood collection methods on experimental outcomes is also discussed.

Additionally, the *British Journal of Pharmacology* provide useful checklists and editorials on reporting animal experimentation (Declaration, 2018; Ingrande et al., 2023; Lilley et al., 2020; McGrath & Lilley, 2015) and design and analysis of experiments (‘Declaration, 2022; Curtis et al., 2022).

2 | BLOOD COLLECTION METHODS

Blood collection in mice is a common procedure applied in a wide range of biomedical research laboratories. Because of their small size, an important parameter to take into consideration is the total volume of blood needed for the experiments. On average, mice have around 58.5 ml of blood per kilogram of bodyweight, and, therefore, a mouse weighing 25 g has a total blood volume of approximately 1.46 ml depending on its age and health status (NC3Rs, 2022b). Depending on the site of collection, a skilled person can collect around 50–75% of a single mouse's total blood volume as part of a terminal, non-recovery procedure, but only 10% of the total volume can be removed every 2 to 4 weeks if the mouse needs to survive the procedure (Lundberg & Skoda, 2011; NIH, 2024; O'Connell et al., 2015; The Jackson Lab, 2005).

Common techniques for blood sampling include collection from the tail vein, saphenous vein, retro-orbital, submandibular (facial) vein, and heart, each with its own advantages and disadvantages (Table 1) (Ahrens Kress et al., 2022; Boston University IACUC, 2021; Hoff, 2000; Lundberg & Skoda, 2011; NC3Rs, 2022b; NIH, 2024; O'Connell et al., 2015; Parasuraman et al., 2010; The Jackson Lab, 2005; Tsai et al., 2015). The site of collection affects the quality and the characteristics of the samples (Ahrens Kress et al., 2022; Tsai et al., 2015). Most of these procedures require analgesia and anaesthesia.

Table 1 provides an overview of the various blood collection techniques, including the route/location, the quantity of blood that can be collected from a specific collection site, the recommended maximal frequency for collection, and any potential drawbacks. Figure 1 provides a decision tree to assist the researcher in determining the most suitable blood collection method based on the required blood volume, frequency, use of anaesthesia and the experimental objective. Table 2 provides an overview of the general guidelines for animal experimentation in UK, Europe, USA, Japan and China. See also the most recent guidelines published by the *BJP* on reporting animal experimentation (‘Declaration, 2020; Ingrande et al., 2023; Lilley et al., 2020; McGrath & Lilley, 2015).

2.1 | How to choose the most appropriate blood collection method?

The method chosen for collecting the blood from experimental mice should be the least painful and stressful for the animal. This is important, not only to comply with the 3R principles and the national regulations (FELASA Working Group, 2023; Gargiulo et al., 2012; GV-SOLAS, 2017; Hoff, 2000; NC3Rs, 2022a; NIH, 2022) (see also Tables 1 and 2) but also because poor sampling technique and unnecessary animal stress may impact on the quality of the samples and the study outcome (Ahrens Kress et al., 2022; Hoggatt et al., 2016; Parasuraman et al., 2010; Rathkolb, Fuchs, et al., 2013; Rathkolb, Hans, et al., 2013; Tsai et al., 2015). Further parameters to consider when choosing a blood collection method are sampling frequency, quality of the sample required (e.g., sterility, disturbance from tissue fluid contamination), personnel proficiency and study requirements.

Depending on the specific requirements of the animal and the experimental conditions, blood collection can be performed with or without anaesthesia and analgesia (FELASA Working Group, 2023; Gargiulo et al., 2012; GV-SOLAS, 2017; Hoff, 2000; NC3Rs, 2022a; NIH, 2022). Importantly, no more than two or three attempts should be made to collect samples from the same site (NC3Rs, 2022a; Parasuraman et al., 2010). Moreover, the researcher should determine in advance the maximum blood volume that can be collected in a single attempt and be aware of the maximum sampling frequency for each site (NC3Rs, 2022a). These two factors should always be considered in combination (NC3Rs, 2022a).

Specifically, SOLAS recommends that no more than 10% of total volume should be collected over a minimum period of 2 weeks, with weekly collection not exceeding 7.5% and the rule of thumb is no

TABLE 1 Overview of the various blood collection techniques, in alphabetical order.

Method	Exp prep/technique	Staff resource	Adverse effects	Amount of blood can be collected	Frequency	Advantages	Disadvantages	Anaesthesia required	References
Blood total amount < 0.2 ml, no anaesthesia required									
Dorsal pedal vein	Mice are kept in a restrainer and the foot is cleaned.	n.a.	n.a.	~0.01 ml. Drops of blood appearing on the skin surface are collected with a capillary tube.	n.a.	n.a.	n.a.	Not requiring anaesthesia.	(Parasuraman et al., 2010)
Saphenous vein	Mice are restrained in a conical tube, clipping the hair, and application of petroleum jelly. Aseptic technique should be used.	One person	Bruising. Haemorrhage. infection. Temporary favouring of the opposite limb.	0.15–0.2 ml (2 weeks interval). 0.01 ml daily.	No more than three attempts can be made: Collecting more than four samples within 24 h not advisable. Microsampling should be considered.	More consistently successful at providing an adequate amount of blood without over-collection as compared to facial and chin bleeding.	Takes longer to collect blood and could cause more stress on mice comparing to facial and chin bleeding; Prolonged restraint and site preparation time.	Not requiring anaesthesia. Local anaesthetic cream may be applied on the collection site.	(DREXEL UNIVERSITY IACUC, 2021; NC3Rs, 2022b; NIH, 2024; Parasuraman et al., 2010)
Sublingual vein	Mice are placed in supine position. The thick caudal part of the right sublingual vein is punctured. Blood is collected into a micro blood collection tube.	Two persons: the second person needs to extend the tongue.	n.a.	0.2 ml	n.a.	Large-volume blood collection with less tissue destruction.	After collection, if animal refuses food for more than 1 day, the animal must be euthanised.	Not requiring anaesthesia.	(GV-SOLAS, 2017; M. Heimann et al., 2009; Maike Heimann et al., 2010; NC3Rs, 2022b)
Submandibular (facial) vein	Mice are restrained by grasping the skin at the back of the neck;use a capillary tube to collect small volumes. Alternatively, collect the drops in a tube as they fall from the puncture site.	One person	Haematoma infection <1%	0.15–0.2 ml	Per face side no more often than once per week.	Repeated sampling is possible by alternating sides of the face. Can be performed rapidly and with minimal amount of equipment, allowing for rapid completion.	Over-collection can occur frequently. Technique limited to adult mice. Sample may be a mixture of venous and arterial blood. Some areas of venous structures in the facial region are covered by other tissues.	Not requiring anaesthesia.	(DREXEL UNIVERSITY IACUC, 2021; Maike Heimann et al., 2010; NIH, 2022, 2024; Queen's University, 2012; Vogt et al., 2022)

(Continues)

TABLE 1 (Continued)

Method	Exp prep/technique	Staff resource	Adverse effects	Amount of blood can be collected	Frequency	Advantages	Disadvantages	Anaesthesia required	References
Tail vein	Mice are kept in a restrainer. Mice may be warmed, to dilate the blood vessel.	One person (mice habituated). Two persons needed for manual restraint.	Low visibility in black or pigmented mice. Infection <1%. Haemorrhage <1%.	≤0.05–0.2 ml. Tail vein sampling is suitable for obtaining ≤0.05 ml (microsample).	Depending on the sample volume, one or two blood samples can be taken per session and within any 24-h period.	From the faecal corticosterone level, the tail vein method is more beneficial compared to the facial vein.	Longer period of time compared to saphenous vein. Could be painful as the tail has ossified vertebral bodies and innervation to the tip. The lack of animal restraint may limit its use to tractable strains of mice.	Not requiring anaesthesia. The use of topical analgesics has been advocated. Local aesthetic cream can be applied on tail 30 min before.	(Abatan et al., 2008; Arras et al., 2007; DREXEL UNIVERSITY IACUC, 2021; Morton et al., 1993; NC3Rs, 2022b; Parasuraman et al., 2010; Whittaker & Barker, 2020)
Blood total amount <0.2 ml, anaesthesia required									
Blood vessel cannulation (carotid artery, vena cava and femoral vein)	Cannulation done under aseptic precautions. Combination of traditional catheterisation system and exteriorised technique	One person takes the blood sample and further staff resource for surgery, post-operative care.	Infection 1–5%. Haemorrhage 1–5%. Blocked cannula 1–5%. Swelling around the jacket 1–5%. Skin sores from the jacket 1–5%.	0.01–0.02 ml	Up to six samples may be taken in a 24-h period. Mice should fully recover and regain body weight 7 days before the next blood collection.	Suitable for use in all strains of mice and good for repeated samples. Less stressful for the animals than repeated vascular punctures.	This method requires close and continuous monitoring of the animal. The cannula requires regular maintenance. Cannulas' length and diameter are important as long cannulas can cause blood to clot and stop the flow.	Surgery is required, and appropriate anaesthesia, analgesia and aseptic technique should be used.	(DREXEL UNIVERSITY IACUC, 2021; GV-SOLAS, 2017; NC3Rs, 2022b; Parasuraman et al., 2010)
Jugular vein	The neck region of the animal is shaved and kept in hyperextended position.	Two persons, one restrains and monitor, one collects blood.	n.a.	30–50 μl	Number of attempts is limited to three.	n.a.	n.a.	Under general/ inhalation anaesthesia or local anaesthetic cream.	(Parasuraman et al., 2010; Shirasaki et al., 2012)
Submental/ chin bleeding	Mice are scuffed and held in dorsal recumbency to expose the chin.	One person	Haemorrhage from the oral or nasal cavity, haematoma formation.	0.1–0.2 ml of blood	n.a.	Suitable for repeated sampling. Fewer externally visible lesions and a lower gross lesion score at	Careful monitoring is needed to make sure animal stopped bleeding before returning it to the cage.	General anaesthesia with isoflurane in an induction chamber.	(UCSF, 2021; Vogt et al., 2022)

TABLE 1 (Continued)

Method	Exp prep/technique	Staff resource	Adverse effects	Amount of blood can be collected	Frequency	Advantages	Disadvantages	Anaesthesia required	References
						necropsy comparing to saphenous site.			
Tail tip amputation/tail snip	Manual restraint of awake animals results in proper site alignment and venous compression for good blood flow. Tail tip amputation more than 5 mm is not acceptable.	n.a.	This method should be avoided as far as possible because it can cause potential permanent damage on the animal tail.	Small volumes from the vein, but medium to large volumes from the artery.	Repeated collections possible. The clot/scab can be gently removed for repeated small samples if serial testing is required.	n.a.	This method causes permanent damage and pain to mice, and the blood might be contaminated with tissue fluid. Hence not recommended. Long-term hyperalgesia after tail-tipping can occur. Sample quality decreases with prolonged bleeding times and 'milking' of the tail.	Before collecting the blood, local anaesthesia is applied on the tail.	(DREXEL UNIVERSITY IACUC, 2021; GV-SOLAS, 2017; NC3Rs, 2022b; NIH, 2022, 2024; Parasuraman et al., 2010; Zhuo, 1998)
Temporary cannula (tail vein)	The animal is restrained. After cannulation, animal must be housed individually in large cages.	n.a.	n.a.	n.a.	n.a.	Tail bleeding normally requires the tail to be warmed up with warm saline to dilate the blood vessels.	n.a.	Local anaesthesia is required.	(Parasuraman et al., 2010)
Blood total amount > 0.2 ml, anaesthesia required									
Abdominal/ thoracic blood vessel	Blood should be withdrawn slowly to prevent the vessel collapsing.	One person	n.a.	0.4–1.0 ml	Once at terminal stage of the study.	Suitable for a single, large, good-quality blood sample. This method can avoid cardiac damage.	n.a.	General anaesthesia is required.	(DREXEL UNIVERSITY IACUC, 2021; NC3Rs, 2022b)
Cardiac puncture	Blood is preferably taken from the ventricle slowly to avoid collapsing of heart.	One person	n.a.	0.1–1.0 ml	Once at terminal stage of the study.	Suitable for a single, large, good-quality blood sample.	Sampling may fail if animal has dextrocardia. It cannot be used if the peritoneum needs to be	General anaesthesia is required.	(DREXEL UNIVERSITY IACUC, 2021; NC3Rs, 2022b; Parasuraman et al., 2010)

(Continues)

TABLE 1 (Continued)

Method	Exp prep/technique	Staff resource	Adverse effects	Amount of blood can be collected	Frequency	Advantages	Disadvantages	Anaesthesia required	References
							lavaged to harvest cells.		
Decapitation	The guillotine should be properly maintained to ensure that it is sharp and functional, and personnel should be properly trained in its use.	One person	n.a.	Up to 1 ml	n.a.	n.a.	Only in rare circumstances under exceptional scientific justification. In UK, personal and project licence authority is required. Blood could be contaminated by body fluids and tissues. A high level of expertise is required for this technique.	Terminal anaesthesia.	(DREXEL UNIVERSITY IACUC, 2021; Mihai Gagea-Iurascu, 2012)
Decapitation (stunning)	Mice being stunned must be dead before decapitation.	One person	n.a.	Up to 1 ml	n.a.	To obtain a large volume of blood that has not been affected by anaesthetic drugs or carbon dioxide.	Only under exceptional scientific justification. Blood could be contaminated by body fluids and tissues.	Not requiring anaesthesia.	(DREXEL UNIVERSITY IACUC, 2021)
Retro-orbital	Mice are restrained, their necks are gently scruffed, and the eye is made to bulge.	One person	Retro-orbital haemorrhage resulting in haematoma and excessive. Pressure on the eye. Penetration of the eye globe itself with a loss of vitreous humour.	0.2 ml (recovery) to 0.5 ml (terminal)	Repeated blood sampling from one eye not earlier than after 2 weeks.	Rapid sampling, high quality of samples.	Higher level of expertise is required. Even a minor mistake will cause damage to the eyes.	General or at least topical ophthalmic anaesthesia. When personnel are undergoing training in retro-orbital blood collection, general anaesthesia is required.	(GV-SOLAS, 2017; NC3Rs, 2022b; NIH, 2022; Parasuraman et al., 2010)

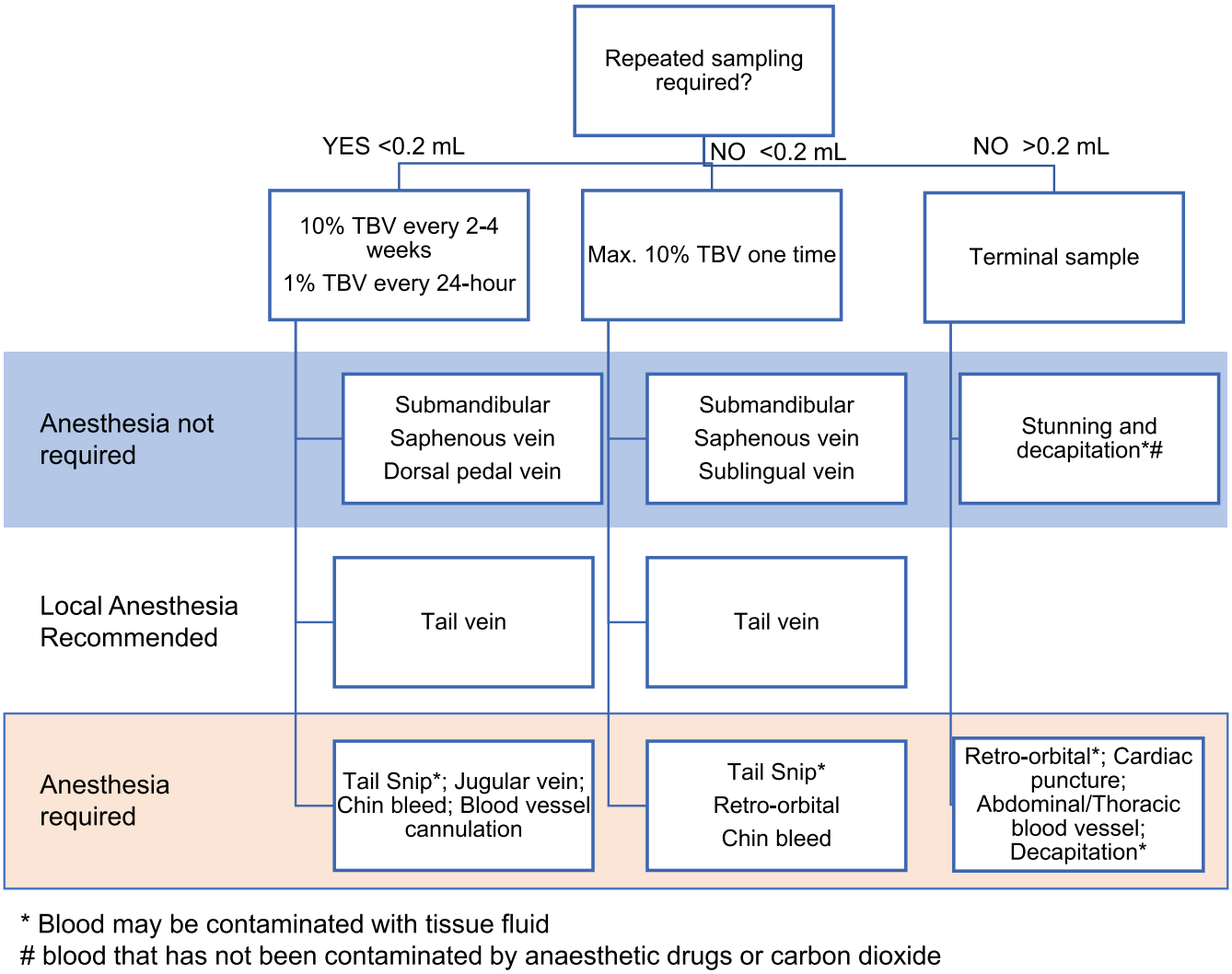

FIGURE 1 Blood collection method decision tree.

more than 1% should be taken daily (GV-SOLAS, 2017). National Institutes of Health (NIH) provides a similar recommendation, which is 10% total volume every 2 to 4 weeks, 7.5% every 7 days and 1% every 24 h (NIH, 2024). If blood collected exceeds 10% of total blood volume of the animal, it is necessary to administer fluid replacement, such a lactate enriched Ringer's solution containing sodium chloride, potassium chloride, calcium and sodium lactate (Pascual et al., 2002; The Jackson Lab, 2005).

If multiple sampling is necessary, catheterisation should be chosen as a less stressful alternative to repeated venipuncture (NC3Rs, 2022a). Instead, exsanguination as a terminal procedure must be performed under general anaesthesia and analgesia. A skilled researcher may collect 50–75% of the total blood volume, which is approximately 1.0–1.5 ml for a 25 g mouse (Diehl et al., 2001).

To reduce the blood sample volumes and the number of small animals used in studies, innovative blood collection methods such as microsampling have been developed (Jonsson et al., 2012; NC3Rs, 2022c; Patel et al., 2016; Wang et al., 2020). Microsampling involves collecting small blood volumes (typically less than 50 µl per time point). Microsampling can be used to determine the haematological profile (such as haematocrit, platelet and white blood cell count) and blood chemistry parameters (such as **glucose**, total **cholesterol** and creatinine) (Chapman et al., 2014; Jonsson et al., 2012; Patel et al., 2016; Wang et al., 2020). This approach is widely used in toxicokinetic and preclinical studies because of its minimal impact on haematological parameters compared to the conventional methods, which usually require 200 µl blood (NC3Rs, 2022c; Patel et al., 2016; Sadler & Bailey, 2013; Wang et al., 2020). With the minimised need for blood volumes, microsampling may reduce or avoid the use of additional set of animals (Jonsson et al., 2012; Wang et al., 2020).

2.2 | Blood collection from the retro-orbital sinus: techniques and animal welfare considerations

The retro-orbital sinus is a commonly used site for both microsampling and collecting larger blood volumes. Retro-orbital blood sampling is fast, which enables blood collection from many animals within a short

TABLE 2 General guidelines in UK, Europe, USA, Japan and China.

Region	Organisation	Regulation/guideline	Common blood collection methods	Additional notes	Ref
UK	ASPA and NC3Rs	Animals scientific procedures act (ASPA), 1986	NC3Rs recommendations – No anaesthesia: saphenous vein, tail vein, mandibular vein, and sublingual vein. With anaesthesia/analgesia: blood vessel cannulation, tail snip, cardiac puncture, abdominal/thoracic blood vessel, retro-orbital and decapitation.	ASPA regulates the handling and surgical procedures on animals carried out for scientific purposes in the UK. Blood collection must be approved by the animal welfare and ethical review body (AWERB). Three licence system: establishment, project and personal licences required. Least painful/stressful methods encouraged. The procedure should be performed by or under the supervision of trained personnel.	(Animals Scientific Procedures Act, 2017; NC3Rs, 2022a, 2022b)
EU	FELASA	Directive 2010/63/EU	GV-SALOS recommendations – 0.01–0.04 ml: tail vein, tail snip, retro-orbital, facial vein and saphenous vein. Single maximum volume: retro-orbital, tail vein, venous angle, and facial vein. Repeated sampling: tail veins (daily), weekly: facial vein, saphenous vein and retro-orbital.Terminal sampling: cardiac puncture, aorta and decapitation.	FELASA aims to implement the 3R principles of laboratory animal science for all procedures regarding experimental animals. A working group is currently evaluating blood sampling methods for rodents, focussing on efficiency, care and research interference. Parameters for blood sampling: Physiologic blood volume, max sample volume, frequency, vessel access and anaesthesia needs. Each country has its own regulations and approval process.	(European Commission, 2024; FELASA Homepage, 2024; FELASA Working Group, 2023; GV-SOLAS, 2017)
USA	APHIS, NIH	Animal welfare act, PHS policy	NIH recommendations – submandibular vein, tail vein, tail clip, saphenous vein and retro-orbital.	Overseen by APHIS under the animal welfare act. IACUCs review protocols to ensure compliance. Use of animal must meet NIH guide for the care and use of laboratory animals and PHS policy on humane care and use of laboratory animals.	(APHIS, 2024; NIH, 2011, 2024; NIH Office of Laboratory Animal Welfare, 2015, 2024)
Japan	JALAS	Law for the humane treatment and management of animals, standards relating to the care and management of laboratory animals and relief of pain	Similar techniques to UK, EU and USA (retro-orbital with welfare considerations).	JALAS was established in 1980. Follows 3R principles and guidelines by the Science Council of Japan. Blood collection requires institutional animal care committee approval, and the procedure has to be carried out exactly as described in protocol with trained personnel.	(JALAS, 2024)
China	CALAS	Statute on the Administration of Laboratory Animal Use	Similar techniques to UK, EU and USA.	CALAS was established in 1987, which oversees animal use under the statute	(CALAS, 2024; Kong & Qin, 2010)

TABLE 2 (Continued)

Region	Organisation	Regulation/guideline	Common blood collection methods	Additional notes	Ref
				approved by the China state council and issued by MOST. PDST administers animal use within each province. Many institutions have IACUCs for project review and compliance with animal welfare principles.	

Abbreviations: ASPA, Animals Scientific Procedures Act; APHIS, Animal and Plant Health Inspection Service; AWERB, Animal Welfare and Ethical Review Body; CALAS, Chinese Association for Laboratory Animal Science; FELASA, Federation of European Laboratory Animal Science Associations; GV-SALOS, Gesellschaft für Versuchstierkunde/Society for Laboratory Animal Science; IACUCs, Institutional Animal Care and Use Committees; JALAS, Japanese Association for Laboratory Animal Science; MOST, Ministry of Science and Technology; NC3Rs, National Centre for the Replacement, Refinement and Reduction of Animals in Research; NIH, National Institutes of Health; PDST, Provincial Department of Science and Technology; PHS, Public Health Service.

timeframe (NIH, 2024). Additionally, the samples generally exhibit good quality with minimal haemolysis or artefactual elevations in biochemical parameters, although contamination with lacrimal fluid can occur (Sharma et al., 2014). For blood volume less than 0.2 ml or 10% or the total blood volume, this method can be used repeatedly by alternating between eyes at intervals of 7 to 14 days, depending on the specific protocol requirements (Boston University IACUC, 2021; Fried et al., 2015; NIH, 2024; Parasuraman et al., 2010). For blood volume of more than 0.2 ml or 10% of the total blood volume, it is a terminal method (NC3Rs, 2022b). When performed by skilled researchers, the incidence of complications is rare, and distress to the animals is minimal (Fried et al., 2015). However, there are some concerns using this method because of its effects on stress and animal welfare (Jo et al., 2021; NC3Rs, 2022b; NIH, 2024; Sharma et al., 2014; Tsai et al., 2015).

The NIH also provides several technical guidelines for this method (NIH, 2024). Sterile capillaries are recommended to help avoid periorbital infection and potential long-term damage to the eye. Alternating orbits should not be performed unless the researcher is proficient in obtaining samples by the dominant hand. The contralateral eye (not being bled) needs to be protected by carefully instilling ophthalmic lubricant without inducing tissue damage by excessive pressure.

Despite its advantages, this method carries potential risks for the animals, including ocular injuries such as blindness, ulcerations, puncture wounds, loss of vitreous humour, keratitis, infections and excessive bleeding, as well as transient pain. Even minor procedural errors can result in severe eye damage (Hoff, 2000; NC3Rs, 2022b; Parasuraman et al., 2010). Therefore, continuous monitoring of animals after the sampling procedure is essential to promptly detect and manage any signs of ocular trauma (Fried et al., 2015). A study has shown that retro-orbital blood collection may induce severe tissue damage and inflammation in the retro-orbital region, characterised by white blood cell infiltration (Jo et al., 2021).

According to the National Centre for the Replacement, Refinement and Reduction of Animals in Research (NC3Rs), retro-orbital sinus blood sampling should only be performed under anaesthesia (NC3Rs, 2022b). The NIH recommends general or at least topical ophthalmic anaesthesia such as proparacaine (proxymetacaine) or tetracaine drops. When personnel are undergoing training in retro-orbital blood collections, general anaesthesia for the animals is required according to the NIH (NIH, 2024), while under UK Animals Scientific Procedures Act (ASPA) legislation, training is not permitted on live animals unless the procedure is part of a study (Animals Scientific Procedures Act, 2017). For further details, Table 2 provides an overview of common blood collection methods as outlined in country-specific guidelines from the UK, Europe, USA, Japan and China.

2.3 | Impact of blood sampling methods on blood count and blood chemistry

It is well known that the animal characteristics (strain, sex, age) as well as the type of anticoagulant (**heparin**, ethylenediaminetetraacetic acid [EDTA], citrate) and post-collection procedures (centrifugation speed, temperature, bench time) crucially affect haematology and blood chemistry (Mazzaccara et al., 2008; O'Connell et al., 2015; Rathkolb, Fuchs, et al., 2013; B. Rathkolb et al., 2013; Silva-Santana et al., 2020). For example, the percentage of neutrophils in blood collected via cardiac puncture in female mice was significantly lower than those in male mice showing that sex steroids have an impact on circulating leukocytes (Doeing et al., 2003).

However, the sampling site can also profoundly influence blood chemistry and cell count (Ahrens Kress et al., 2022; Gjendal et al., 2020; Hoggatt et al., 2016; Meyer et al., 2020; Nemzek et al., 2001). Interestingly, red blood cell count appears to be higher if blood samples are derived from more peripheral sites as compared to the heart (Abatan et al., 2008; Nemzek et al., 2001). One study reported that the blood cell counts were the highest from tail blood, lower in retro-orbital blood and the lowest if blood taken from the heart (Nemzek et al., 2001). Additionally, higher haematocrit was observed in tail blood compared to blood taken from retro-orbital or from the heart.

Instead, white blood cell counts appear to gradually decrease from the periphery to the heart (Doeing et al., 2003; Goldie et al., 1954; Hoggatt et al., 2016). Compared to cardiac puncture, retro-orbital sinus, facial vein or tail bleeding blood sampling yielded a lower proportion of dendritic cells, indicating its unsuitability for isolating these cells (Hoggatt et al., 2016). In the same study, they observed that the haematopoietic progenitor cell count was also affected by the sampling site, being significantly higher in tail blood.

Modifying experimental conditions such as the temperature of the site of collection or animal acclimatisation may also result in different outcomes (Doeing et al., 2003; Hoggatt et al., 2016; Marin et al., 2023). Warming of the tail prior to tail bleeding resulted in significantly more myeloid-derived suppressor cells and lower numbers of peripheral haematopoietic progenitor cells compared to samples obtained without pre-warming the tail (Hoggatt et al., 2016). Animal acclimatisation prior to blood sampling significantly reduced glucose levels and faecal corticosterone metabolites, likely related to a reduction in stress response for the mice (Marin et al., 2023).

2.4 | Summary

Taken together, the blood sampling method, collection site and experimental conditions (including the use of anticoagulants and post-collection procedures) strongly affect experimental results. Therefore, in addition to animal-specific characteristics, the chosen blood collection method and sites must be carefully considered during experimental design and kept consistent throughout the procedures. See also the most recent guidelines and checklists for experimental design published by the *British Journal of Pharmacology* (Curtis et al., 2022; Declaration of transparency and scientific rigour: Checklist for Design and Analysis 2022, Declaration, 2022).

3 | METHODS FOR ANAESTHESIA AND ANALGESIA

Pain and distress increase the release of stress hormones such as **adrenaline** and **cortisol** (Ahmadi-Noorbakhsh et al., 2022; Gong et al., 2015), which in turn influence cardiovascular and renal parameters including heart rate, cardiac output and glomerular filtration rate. Proper use of anaesthesia and analgesia can efficiently prevent these stress responses, produce more consistent data, and potentially reduce the number of animals needed for the experiments (Reduction, Refinement) (Ahmadi-Noorbakhsh et al., 2022; Gargiulo et al., 2012). Sex, strain, body weight and genomic alterations may affect sensitivity to certain anaesthetics and thus need to be taken into consideration by the choice of analgesia and/or anaesthesia (Gargiulo et al., 2012).

Pre-anaesthetic care is an important part of the anaesthesia procedure and can reduce the complications and variables during anaesthesia (Ahmadi-Noorbakhsh et al., 2022; Gargiulo et al., 2012; Navarro et al., 2021). Animal handling and restraint should be non-aversive when possible (see, for example, NC3Rs mouse handling guidance [NC3Rs, 2023]). Physical restraint and manipulation may affect physiological functions and stress response hormones, leading to the need for higher anaesthetic induction doses (Gargiulo et al., 2012; Hildebrandt et al., 2008).

The anticholinergic **muscarinic** antagonist drug **atropine** (0.04 mg kg^{-1} s.c., i.p., i.m.) is commonly used as a pre-anaesthetic medication for various species to minimise salivation, bronchial secretions, and protect the heart from vagal inhibition by anaesthetics like **xylazine** (Gargiulo et al., 2012). In cases where animals are particularly agitated, mice can be treated with a lower dose of a phenothiazine tranquilliser like acepromazine (2–5 mg kg^{-1} i.p) before or in combination with anaesthetics like **ketamine**/xylazine (Ahmadi-Noorbakhsh et al., 2022). However, the potential side effects and the possible interference of these drugs with experimental results should also be carefully considered. Factors such as altered physiological parameters, delayed recovery times and drug interactions may influence the reliability of the collected data. Therefore, selecting appropriate doses, administration routes and combinations should be guided by both experimental requirements and animal welfare considerations.

Mice are particularly sensitive to changes in temperature (Gong et al., 2015; Hankenson et al., 2018). During anaesthesia, the animal should be kept in a warm environment by using heating pads or infrared lamps to avoid hypothermia and temperature effects on blood sampling (Hankenson et al., 2018). Furthermore, fluid therapy following the blood sampling procedure facilitates a fast and complete recovery from anaesthesia. Indeed, administration of 0.9% saline or half-strength dextrose/saline solution (1.2 ml divided in two daily doses) may be administered s.c for preventing dehydration in mice (Gargiulo et al., 2012).

Full-body anaesthesia regimens for mice can be administered either by injection or inhalation, using a mask or intubation for more stable anaesthesia. If the chosen anaesthetic lacks analgesic properties, appropriate analgesic medication should be administered to alleviate pain. The depth of full body anaesthesia can be assessed by testing the palpebral reflex or the pedal reflex (Ahmadi-Noorbakhsh et al., 2022; Gargiulo et al., 2012; Navarro et al., 2021). When applicable, local anaesthesia can be used for specific blood collection procedures that do not require full-body anaesthesia (Table 1). According to the ASPA recommendations, if anaesthesia is not possible, analgesics or other appropriate methods should be used in order to ensure that in any event the animal is not subject to severe pain, distress or suffering (Animals Scientific Procedures Act, 2017).

The use of anaesthetics might affect blood parameters. For example, xylazine is likely to induce hyperglycaemia because it inhibits **insulin** secretion on pancreatic islets. Additionally, slight to moderate increases in glucose were observed in **isoflurane** (2.5%) anaesthetised animals used for submandibular blood sampling when compared with conscious animals (Maike Heimann et al., 2010).

Some common general and local anaesthesia and analgesia methods for blood collection in mice are described in the following paragraphs and summarised in Table 3. See also the recommendation for the use and reporting of anaesthesia in mice in the *British Journal of Pharmacology* (Ingrande et al., 2023).

TABLE 3 Commonly used analgesics and anaesthetics.

Drug	Purpose	Doses	Application	Notes
Proparacaine	Anaesthesia	0.5% ophthalmic solutions	Local (ophthalmic)	Applied before retro-orbital blood sampling
Tetracaine	Anaesthesia	0.5% ophthalmic solutions	Local (ophthalmic)	Applied before retro-orbital blood sampling
Lidocaine + Prilocaine (EMLA cream)	Anaesthesia	25 mg g^{-1} lidocaine + 25 mg g^{-1} Prilocaine (topical)	Local (topical)	Applied to the tail tip before sampling
Butorphanol	Analgesia	1–5 mg kg^{-1}	Systemic, s.c., or i.p. injection (analgesic)	Pre-emptive use reduces intraoperative pain
Isoflurane	Anaesthesia	4–5% for induction, 1–3% for maintenance	Systemic (inhalation)	Short induction and recovery time; suppresses respiration, no analgesic effect
Sevoflurane	Anaesthesia	4–5% for induction, 1–3% for maintenance	Systemic (inhalation)	Faster induction and recovery than isoflurane
Buprenorphine	Analgesia	0.1 mg kg^{-1} i.p. (supplementary analgesia). 0.05–0.1 mg kg^{-1} s.c. (post-operative analgesia every 6–12 h. Extended-release formulation: 1–2 mg kg^{-1} s.c. every 48–72 h.	Systemic (i.p.)	Required for supplementary analgesia when used with isoflurane
Ketamine + xylazine	Anaesthesia	Ketamine: 80–200 mg kg^{-1}; xylazine: 5–20 mg kg^{-1} i.p.	Systemic (i.p)	Provides good safety margin; may cause hypertension or hypotension; increase mortality in aged mice.
Pentobarbital	Anaesthesia	40–50 mg kg^{-1} i.p.	Systemic (i.p.)	May induce abdominal pain if not buffered; requires caution
Thiopental	Anaesthesia	30–40 mg kg^{-1} i.v.	Systemic (i.p.)	Rapid action; risk of respiratory and cardiovascular depression

3.1 | Local anaesthesia

Local anaesthetics are used to reduce discomfort and stress during blood sampling procedures from the tail tip and are also recommended during retro-orbital sinus blood collection. For example, the eutectic mixture of local anaesthetics (EMLA) cream (25 mg g^{-1} **lidocaine** + 25 mg g^{-1} **prilocaine**) can be applied to the tail tip before sampling (Arras et al., 2007).

Topical ophthalmic anaesthetic like **proparacaine** or tetracaine drops need to be applied to the eye prior to blood collection from the retroorbital sinus to provide sufficient analgesia (Ahmadi-Noorbakhsh et al., 2022; see also Section 2.2).

3.2 | General anaesthesia

Isoflurane (3.5–5.0% for induction, 1–3% for maintenance) is a volatile anaesthetic used for both short and long procedures in animals (but also in humans), which is administered to the mice via inhalation by using a mask or by intubation (Brunson, 1997; Diven, 2003; Gargiulo et al., 2012; Navarro et al., 2021). The advantages of isoflurane (and in general of all volatile anaesthetics) are that they are rapidly distributed, quickly cross the blood–brain barrier and are eliminated as quickly via expiration. Moreover, isoflurane is characterised by a short induction and recovery time (Diven, 2003). The main disadvantages include its potent suppression of the respiratory system and its vasodilatory effects. It lacks analgesic effects, so it requires additional administration of analgesia like **buprenorphine** (0.1 mg kg^{-1}, i.p.) to be administered 10 min before induction of anaesthesia (Ahmadi-Noorbakhsh et al., 2022; Diven, 2003). It was found to elevate blood glucose concentrations (Maike Heimann et al., 2010; Marquardt et al., 2018). **Sevoflurane** offers more control (faster induction and recovery), but whether or not it is less aversive to rodents is still under debate (Brunson, 1997; Guedes et al., 2017; Marquardt et al., 2018; Otto & von Thaden, 2012).

Barbiturates frequently used in mice are **thiopental** (30–40 mg kg^{-1} i.v.) and **pentobarbital** (40–50 mg kg^{-1} i.p.) (Otto & von Thaden, 2012), but the dose is dependent on strain and other animal characteristics (sex, weight and age). It is important to note that i.p. pentobarbital induces abdominal pain if not buffered prior to administration, but this is not an issue if given i.v. All barbiturates share the advantages of long shelf life and rapidity of action (Brunson, 1997; Otto & von Thaden, 2012). The primary adverse effects include respiratory and cardiovascular depression. Injections of higher doses of barbiturate are also used for euthanasia (Pang & Laferriere, 2020).

The administration of a combination of xylazine and ketamine is also a common anaesthesia method in mice. Ketamine is a dissociative agent, and xylazine is an **α_2-adrenoceptor** agonist muscle relaxant. The doses of ketamine and xylazine depend on the mouse strain, with the range of xylazine being 5–20 mg kg^{-1} (Erhardt et al., 1984; Hart et al., 2001) and the range of ketamine being 80–200 mg kg^{-1}

(Buitrago et al., 2008). This combination provides a good safety margin with a certain level of analgesia. However, the distinctive side effects when administering α_2-adrenoceptor agonists such as xylazine include hypertension, hypotension, elevated peripheral vascular resistance and diminished cardiac output (Brunson, 1997; Gargiulo et al., 2012; Otto & von Thaden, 2012). It is reported that the mortality rate increases in aged mice using this anaesthetic method (Schuetze et al., 2019). Depths and duration of anaesthesia obtained by combining ketamine with α_2-adrenoceptor agonists are variable; therefore, it is may be supplemented with analgesics such as **butorphanol** (Bauer et al., 2019).

3.3 | Summary

Taken together, analgesia and anaesthesia methods are essential for selected blood sampling procedures in mice to ensure animal welfare and data reliability (Table 3; see also Ingrande et al., 2023). The most commonly used anaesthetics include inhalation agents such as isoflurane and sevoflurane, which offer precise anaesthetic depth control with rapid onset and recovery. Injectable anaesthetics like ketamine–xylazine provide deeper sedation for invasive procedures but require careful monitoring because of potential respiratory and cardiovascular effects. Local anaesthetics such as proparacaine and tetracaine are effective for procedures involving the eyes, while opioid analgesics like buprenorphine and butorphanol manage post-operative pain. Selecting the appropriate method depends on the procedure's invasiveness, duration and expected pain level, ensuring both scientific accuracy and ethical responsibility.

4 | DOCUMENTING AND REPORTING BLOOD TAKING PROCEDURES AND ANAESTHESIA

Documenting and reporting any experimental procedure is critical for ensuring the validity and reliability of experimental results. Table 4 outlines the key aspects of documenting and reporting blood collection and anaesthesia procedures. See also the most recent guidelines published by the *BJP* for reporting animal experiments according to ARRIVE guidelines and the use of anaesthetics (Curtis et al., 2022; Ingrande et al., 2023; McGrath et al., 2010; McGrath & Lilley, 2015; Percie du Sert et al., 2020; Stanford et al., 2023).

For reporting the experiments involving animals, it is essential to provide species-appropriate details, including strain, sex, weight and age (Stanford et al., 2023). Further relevant information on the provenance, health and immune status, genetic modifications, and genotype of the animals should be reported as well. These variables impact animal welfare, experimental consistency, scientific outcomes and reproducibility (Percie du Sert et al., 2020). For reporting genotype and strains, the Jackson Laboratory issued a guideline for standardised genetic nomenclature (The Jackson Lab, 2024).

TABLE 4 Reporting blood collection, anaesthesia and analgesia protocols.

Items to report	Details
Ethical statement	The ethical review committee or equivalent that has approved the use of animals in this study. If not, provide justification.
Mouse details	Source, strain, sex, weight, age.
Further relevant animal information	Provenance, health/immune status, genetic modification status, genotype and any previous procedures.
Blood collection procedure	Site, volume of blood, anticoagulant, frequency, aseptic techniques, handling, rationale and justification of the choice.
Anaesthesia and analgesia	Drug type, formulation, dose per kilogram, volume, concentration, administration site and route, frequency, and duration, pre- and post-analgesia regimen, rationale and justification for the choice.
Welfare assessment	Expected or unexpected adverse events, signs of actual distress, changes in animal behaviour, humane endpoints for the study, signs monitored and frequency of monitoring, score sheet system.
Method of euthanasia	For animal that reached an humane end point.

Blood collection procedures require continuous monitoring of animals and their environment throughout the experiment. An initial assessment should document key parameters, including behaviour, body condition, respiratory rate, food and water intake, and signs of illness such as skin lesions, discharge or perineal soiling. Monitoring and documenting should continue, as signs of disease may emerge at later stages, often during anaesthesia, or in the post-anaesthetic recovery phase. Animals that have reached humane endpoints should be killed to minimise harm. Also, the method of euthanasia (performed according to local guideline, e.g. see the EU guidelines [European Commission: Directorate-General for Environment et al., 1997] and the American Veterinary Medical Association [AVMA] guidelines in USA) (AVMA, 2020; NIH OACU, 2024), pharmacological agent, any measures taken to reduce pain and distress before or during the procedure, and the time point should be documented.

For any studies, the *humane endpoints*, along with the *clinical signs* observed and the *frequency of monitoring*, need to be defined in advance. For this purpose, a *score sheet system* is commonly used to assess health status and to pre-define the clinical sign and humane endpoints. Publishing the score sheets of the clinical signs that were monitored can help guide other researchers to develop clinically relevant welfare assessments. The NIH Office of Animal Care and Use (OACU) suggested a list of items to be considered when developing and evaluating justifications for studies involving increased levels of pain and/or distress (NIH OACU, 2024).

Specifically for blood collection procedures, the following details need to be documented and reported for assuring scientific reproducibility and transparency of animal procedures: a description of the

procedure itself (blood collection site, volume of blood and anticoagulant), specifics regarding anaesthesia (including dosage, drug type, formulation, volume, concentration, administration site and route, frequency and duration), pre- and post-analgesia regimen, aseptic techniques employed, monitoring procedures, clarification on whether the procedure is terminal, its duration and the physical variables being measured. Timing and frequency of blood collection also need to be documented for welfare assessments. It is important also to indicate whether mice were allowed to acclimatise after stressful events like transport or handling procedures. Moreover, a short explanation of the rationale behind the chosen procedure or technique could improve the quality of documentation and the transparency of the study (Percie du Sert et al., 2020). Researchers should also ensure the procedures they choose comply with the animal legal framework of the countries where they are carried out and published (J. McGrath et al., 2010).

A detailed and effective reporting of animal anaesthesia and analgesia procedure includes the specific analgesic, administration method, rationale for the choice and protocol modifications (Percie du Sert et al., 2020).

Taken together, detailed documentation of experimental procedures with standardised nomenclature of animal characteristics, anaesthesia, or euthanasia methods, along with the rationale behind the protocol, is crucial for study transparency, reliability, reproducibility and the development of welfare assessments.

5 | SUMMARY

Selection of a blood collection method in mice involves balancing between scientific needs and ethical consideration of animal well-being. Each method has its own set of advantages and limitations, and the choice should be made with careful consideration of the study objectives and the potential impact on the animal, in consultation with the resident or named veterinarian. Adequate training and expertise in the chosen method are essential to ensure both animal welfare and the quality of the collected samples. Additionally, when required by the procedure, mice should undergo appropriate anaesthesia and/or analgesia to minimise pain and distress while maintaining validity of sample collection. The choice of blood collection method and anaesthesia should consider factors such as animal welfare, sample volume and frequency. Furthermore, comprehensive reporting routines and score sheets are helpful and necessary in order to improve transparency and reproducibility in any experimental procedure. Ultimately, these standardised practices aim to improve data reproducibility and support ethical animal research.

5.1 | Nomenclature of targets and ligands

Key protein targets and ligands in this article are hyperlinked to corresponding entries in http://www.guidetopharmacology.org, and are permanently archived in the Concise Guide to PHARMACOLOGY 2021/2022.

AUTHOR CONTRIBUTIONS

Zhixin Li: Conceptualization (equal); investigation (equal); visualization (lead); writing—original draft (lead). **Anthea LoBue:** Writing—review and editing (supporting). **Sophia K. Heuser:** Writing—review and editing (supporting). **Junjie Li:** Writing—review and editing (supporting). **Eva Engelhardt:** Writing—review and editing (supporting). **Andreas Papapetropoulos:** Writing—review and editing (supporting). **Hemal H. Patel:** Writing—review and editing (supporting). **Elliot Lilley:** Writing—review and editing (supporting). **Péter Ferdinandy:** Writing—review and editing (supporting). **Rainer Schulz:** Writing—review and editing (supporting). **Miriam M. Cortese-Krott:** Conceptualization (equal); funding acquisition (lead); project administration (lead); resources (lead); supervision (lead); writing—review and editing (supporting).

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CONFLICT OF INTEREST STATEMENT

PF is the founder and CEO of Pharmahungary Group, a group of R&D companies.

DATA AVAILABILITY STATEMENT

N/A-Review.

DECLARATION OF TRANSPARENCY AND SCIENTIFIC RIGOUR

This Declaration acknowledges that this paper adheres to the principles for transparent reporting and scientific rigour of preclinical research as stated in the *BJP* guidelines for Design and Analysis, and as recommended by funding agencies, publishers and other organisations engaged with supporting research.

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