Heart Ventricular Progenitor Cell Therapy and Using Gottingen Minipigs for Development of Curative Cardiovascular Cell Therapies

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Points Covered in the Presentation

Overview of the cell therapy

- Use of cells in curative treatments
- Heart Ventricular Progenitor Cells (HVP) in heart failure

Study Designs and Considerations

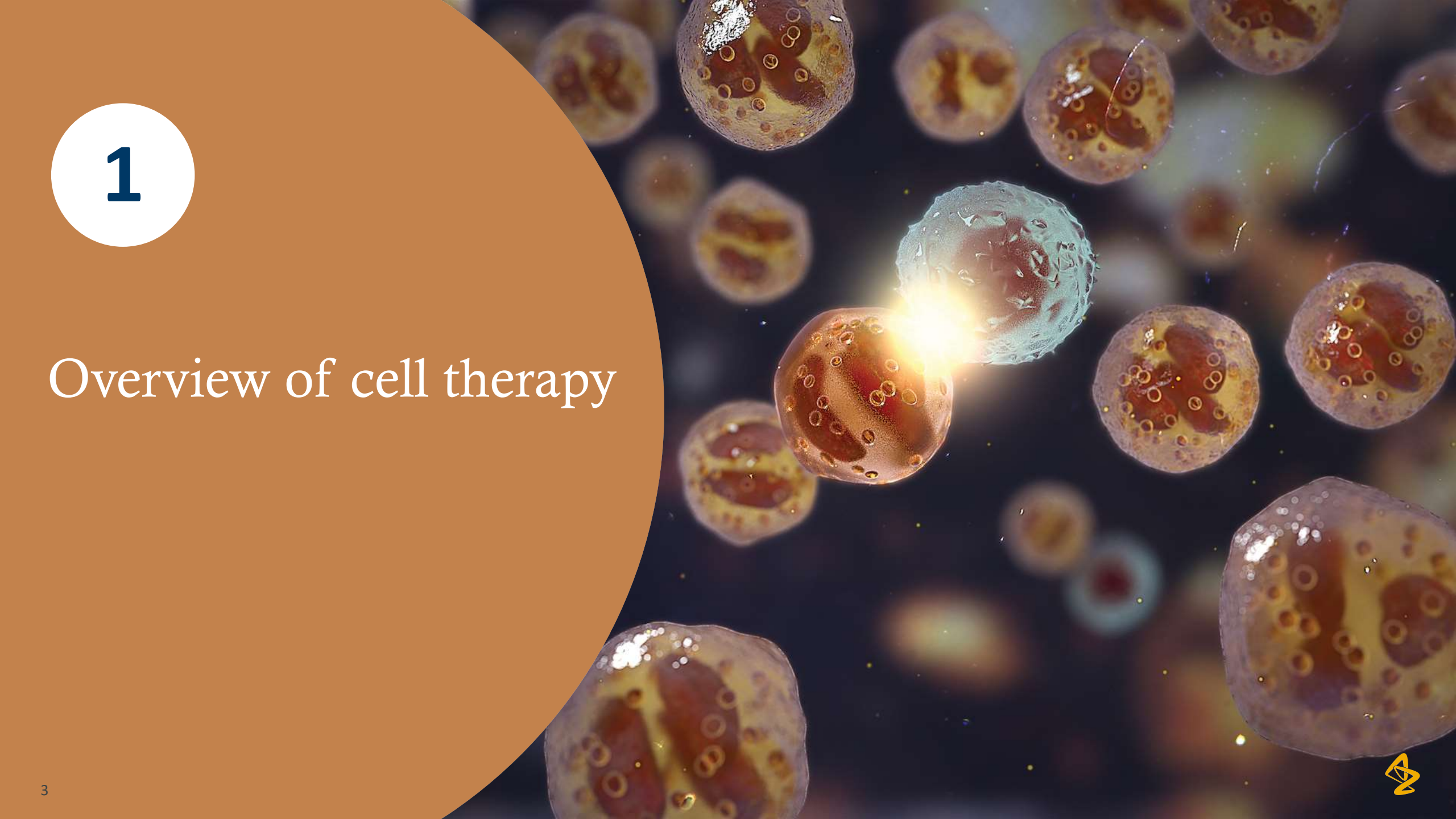
- Considerations and overview of the practical study challenges
- Examples of data from studies

Immunosuppression and Challenges

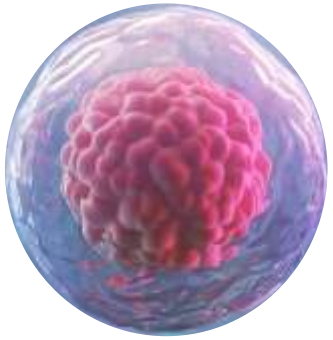
- Details of immunosuppression and co-medication regimens
- Challenges of using immunosuppression

1

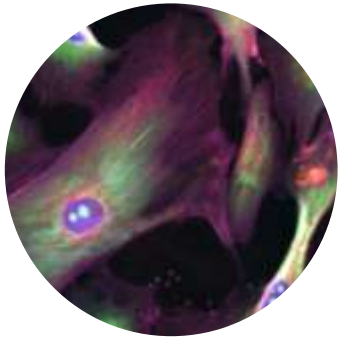
Overview of cell therapy



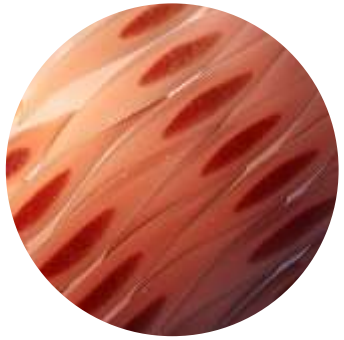
Using cells to develop a curative treatment for heart failure patients



The **right cell**, differentiating to mature cardiomyocytes



Sufficient number of cells engrafting for durable efficacy of product



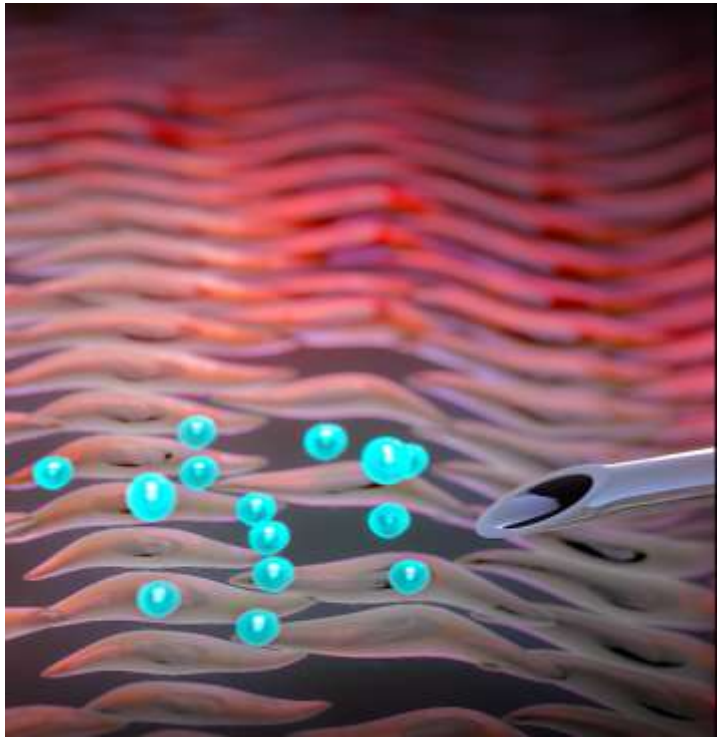
Pure cell population without risk for teratoma

Robust QC and CMC processes for reproducibility of product

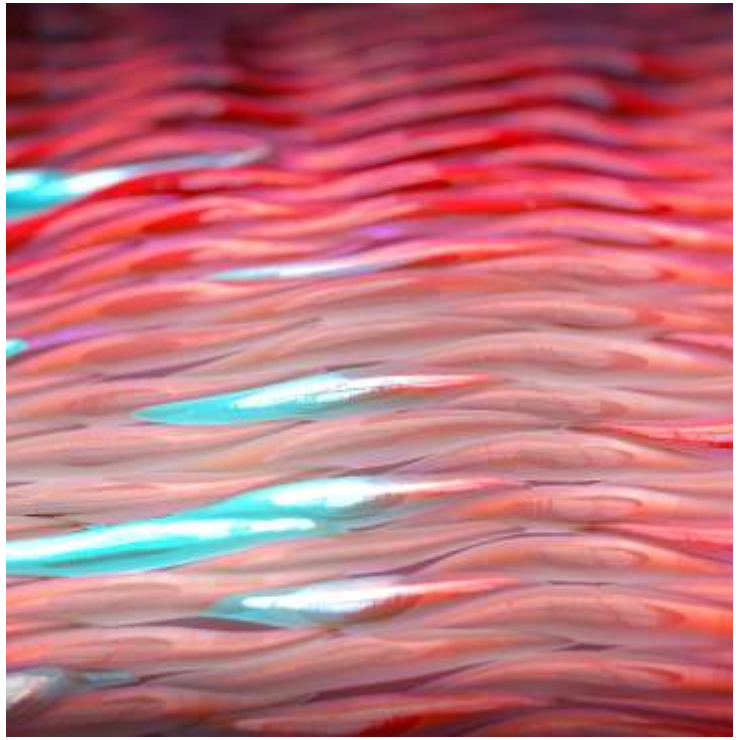
HVPs as a regenerative cell therapy in heart failure

HVP cells are injected in the heart…

…and differentiate and integrate…

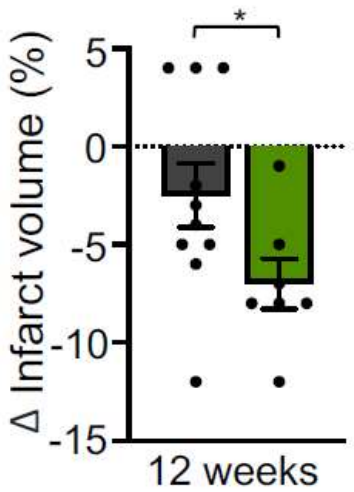


…and regenerate new functional cardiac tissue

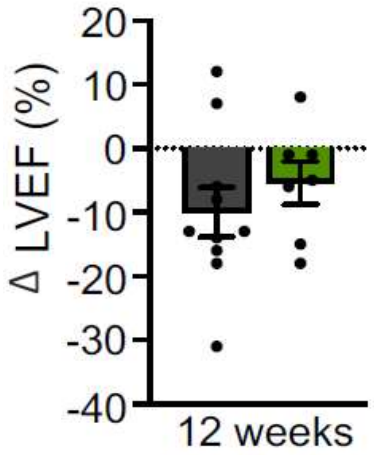


HVPs reduce infarct size and attenuate decline in cardiac function three months after injection in post myocardial infarction minipigs

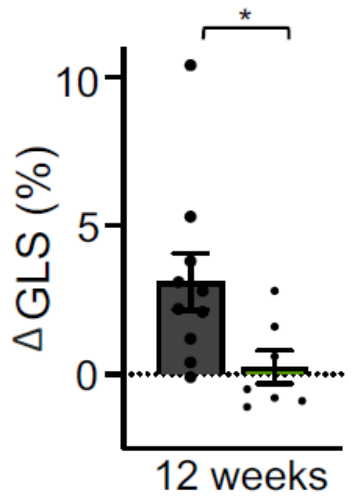
Infarct volume



Ejection fraction



Global longitudinal strain

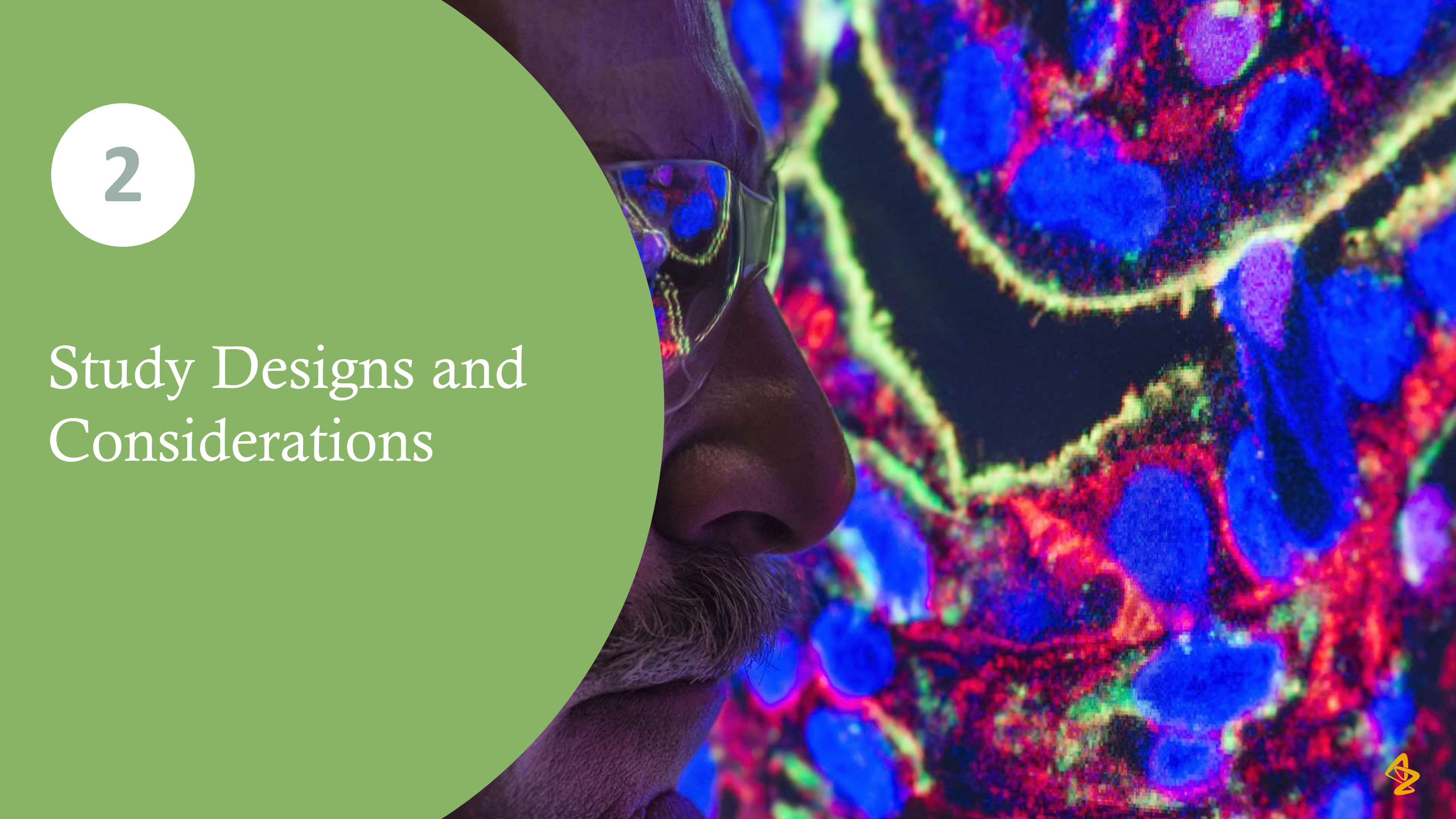


vehicle group HVP group

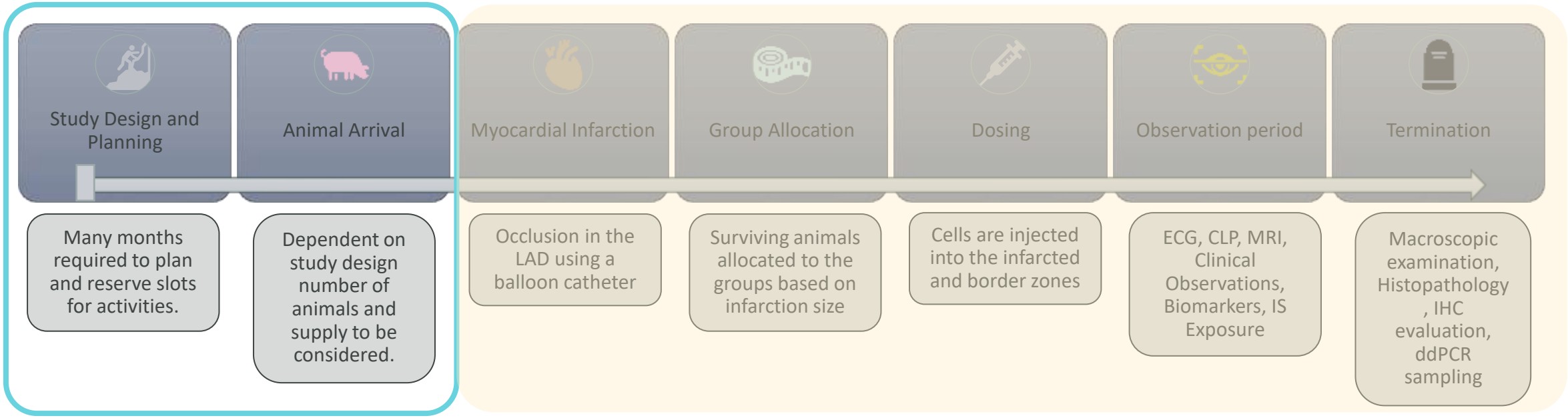
Procella THERAPEUTICS

2

Study Designs and Considerations

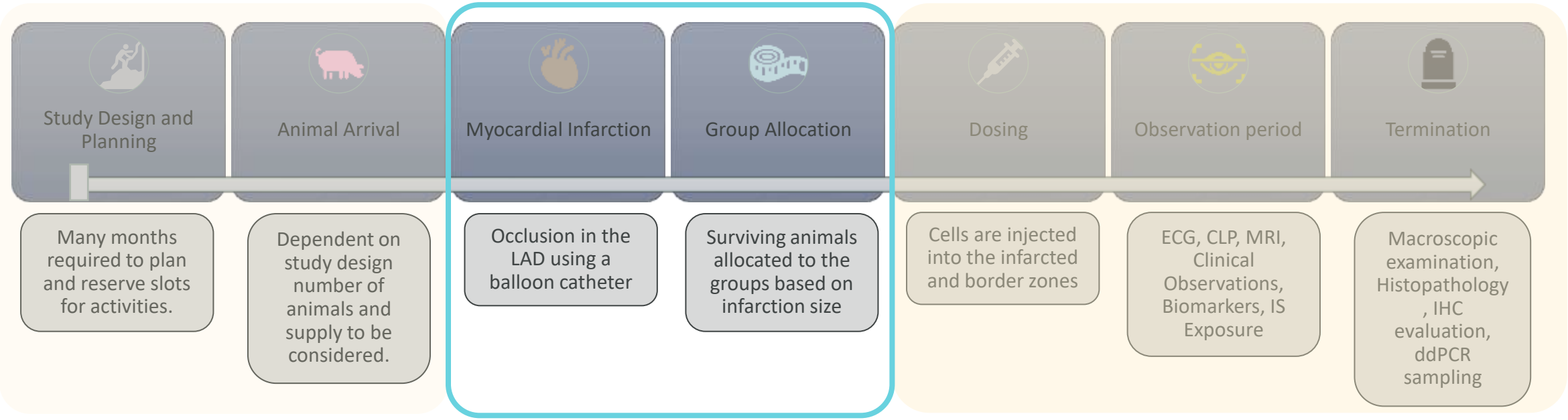


Study design and animals dependent on objectives of the study



- Animal number and housing requirements
- Surgery slots
  - Myocardial infarction procedures – understanding the technique and identifying success rates
  - Securing surgery resources to perform the surgeries
  - Inclusion of spare slots for possible replacement animals
- Dose levels
  - Cell handling – cell thaw and resuspending protocols
  - Recovery expectations
- Animal logistics and welfare aspects:
  - Pre and post surgery care
  - Pre-approved treatments and medication for emergency use
  - Endpoint clarification
- Veterinarian care plan and partnership

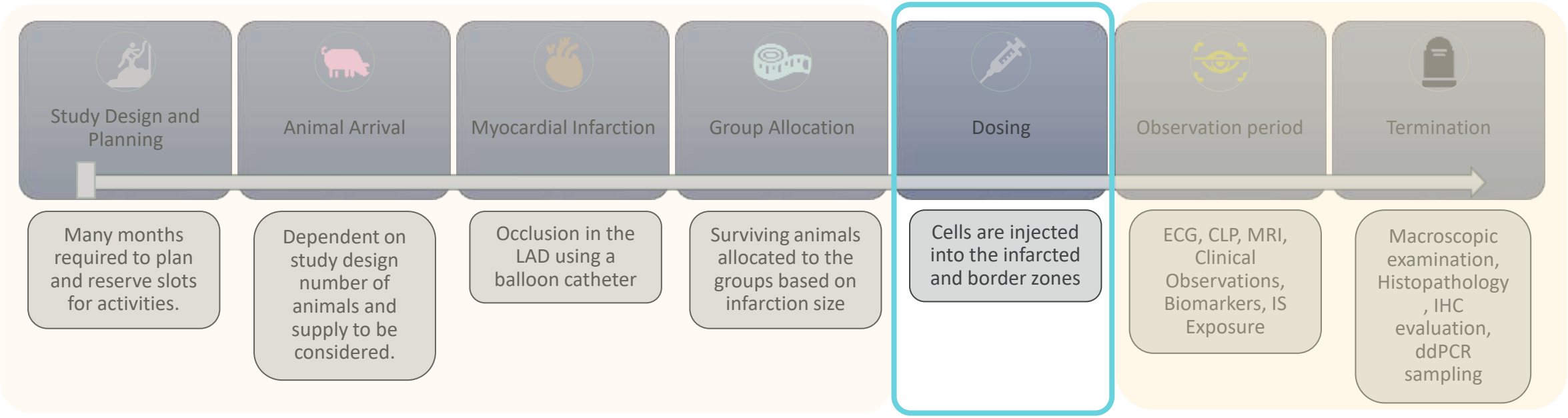
Myocardial infarction (MI) and group allocation considerations



- MI procedure is specialised and requires an experienced surgical team
- Placement of the occlusion balloon in the LAD is important to obtain a sufficient infarct
- Large infarctions to ensure study enrolment but has risks to the animal

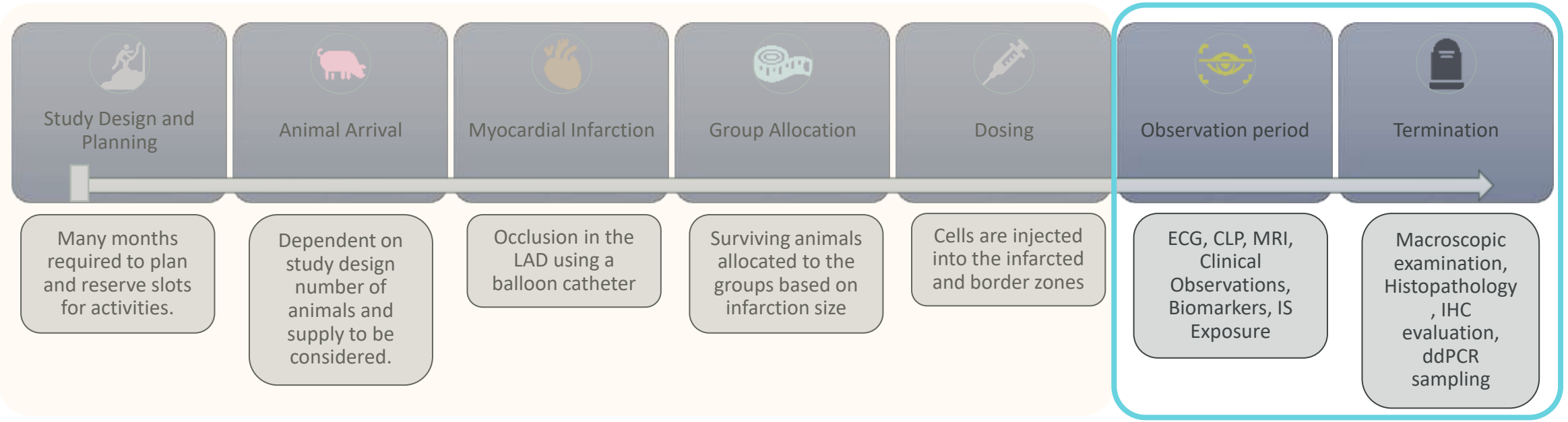
- ECHO or MRI measurements to obtain ejection fraction (EF) %
- Allocation criteria: EF% or percentage change from the baseline EF% will be used to allocate animals onto the study and within groups
- Randomisation of animals into groups is challenging as it is dependent on the number of available for allocation and
- Dimensions of the infarction need to be provided prior to surgery understand the nature of the infarction and initial distribution of injections

Dosing considerations



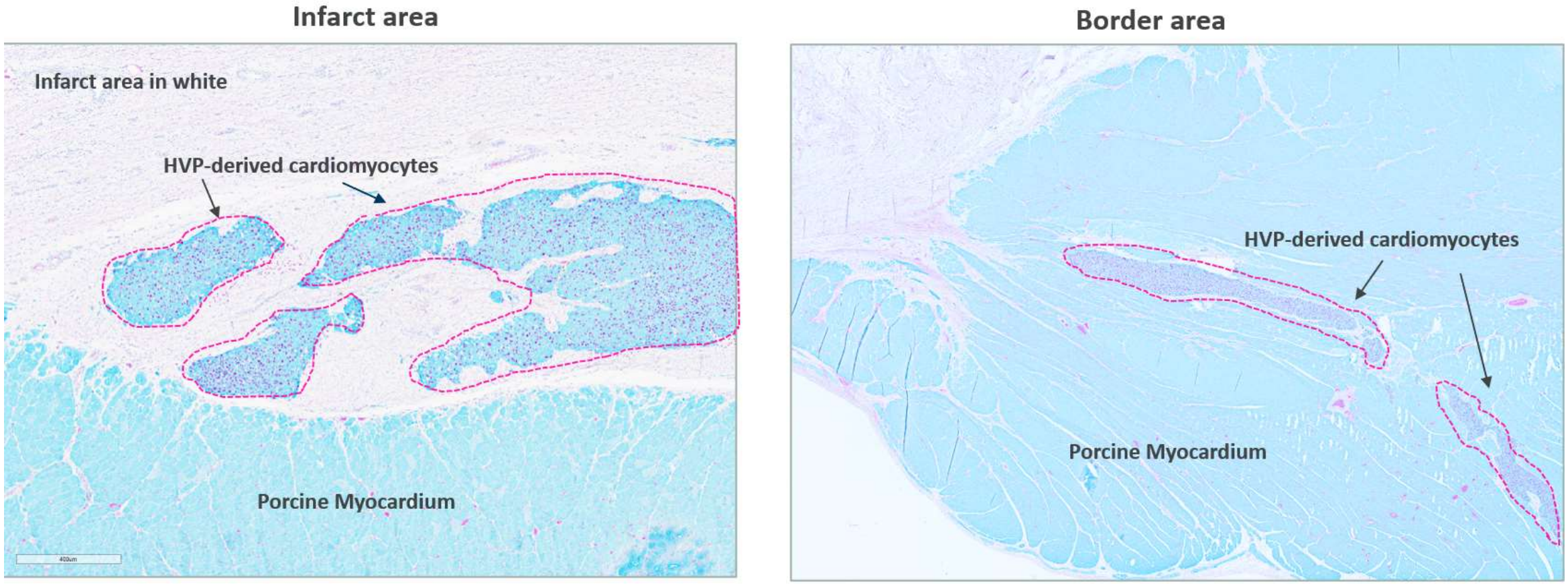
- Surgical procedure (sternotomy) is very risky for the animal
  - Incision in the skin over your breastbone (sternum) and cut through your sternum
- High risk of infection
- Pre-approved veterinarian intervention treatment plans required
- Robust care and welfare plans to ensure appropriate and high level welfare is maintained
- Number and placement of the injection important for successful engraftment and obtaining endpoints
- Identification of the injections within the heart required
- High quality dosing records required to ensure correct areas are processed for histopathological endpoints

Data assessment during the observation period and termination



- In life updates on the data collected important for understanding the success of the dosing:
  - ECG measurements soon after collection
  - Immunosuppression exposure levels
  - MRI measurements
- Clinical observations during this period is critical for maintaining good animal welfare and being reactive to possible adverse events
- Collection of the heart tissue for standard histopathological evaluations
- Correct areas collected to assess cell engraftment and integration - IHC
- Tissue collection for biodistribution characterisation - PCR

HVP cells remuscularize the myocardium and form grafts in infarct and border area 3 months after injection in minipigs

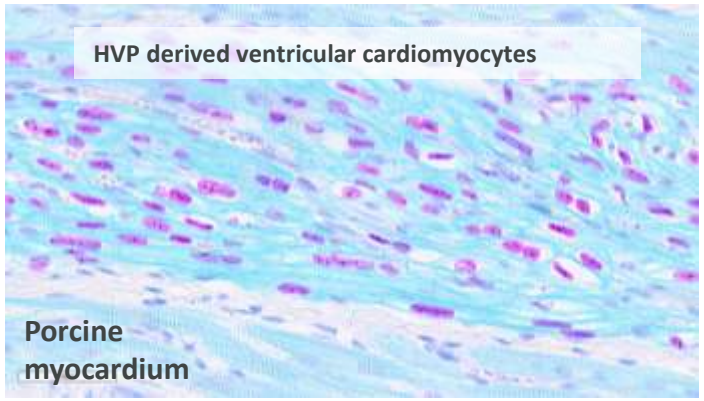


Cardiac Troponin T (Cardiomyocytes) – Human Nucleoli (HVP- derived cardiomyocytes)

Marcella Sini, Magnus Söderberg

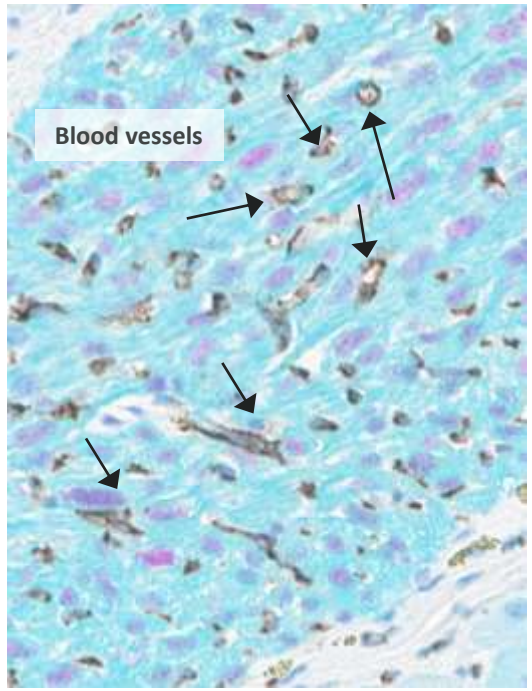
HVP cells differentiate to ventricular cardiomyocytes and connect with host tissue in pig heart 3 months after myocardial infarction

Formation of ventricular cardiomyocytes



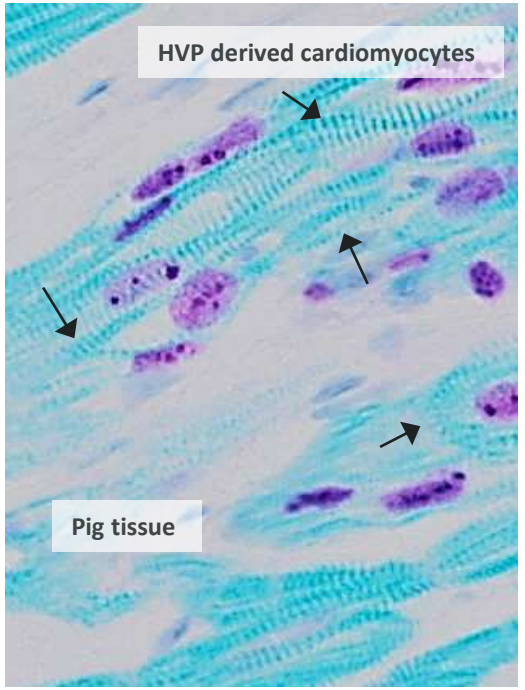
MLC2v (ventricular cardiomyocytes), Human Nucleoli (HVP derived cardiomyocytes)

Vascularization



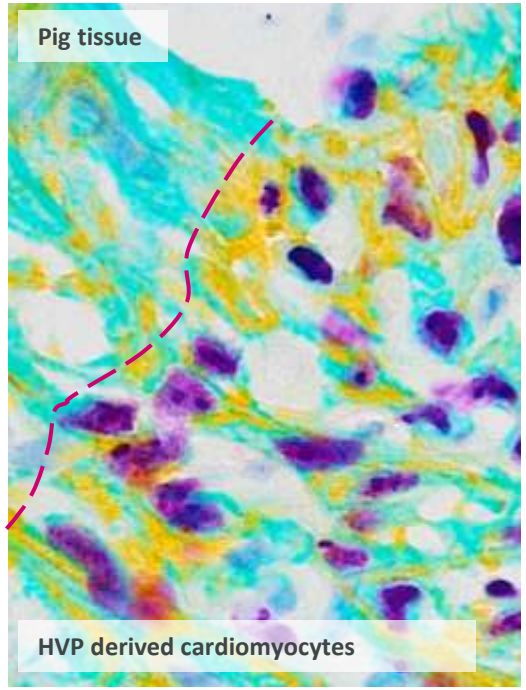
CD31 staining (pig and human endothelial cells) MLC2v (ventricular cardiomyocytes) Human Nucleoli (HVP derived CM)

Sarcomer formation



A-actinin (striation in CM) Human Nucleoli (HVP derived CM)

Connection with host tissue

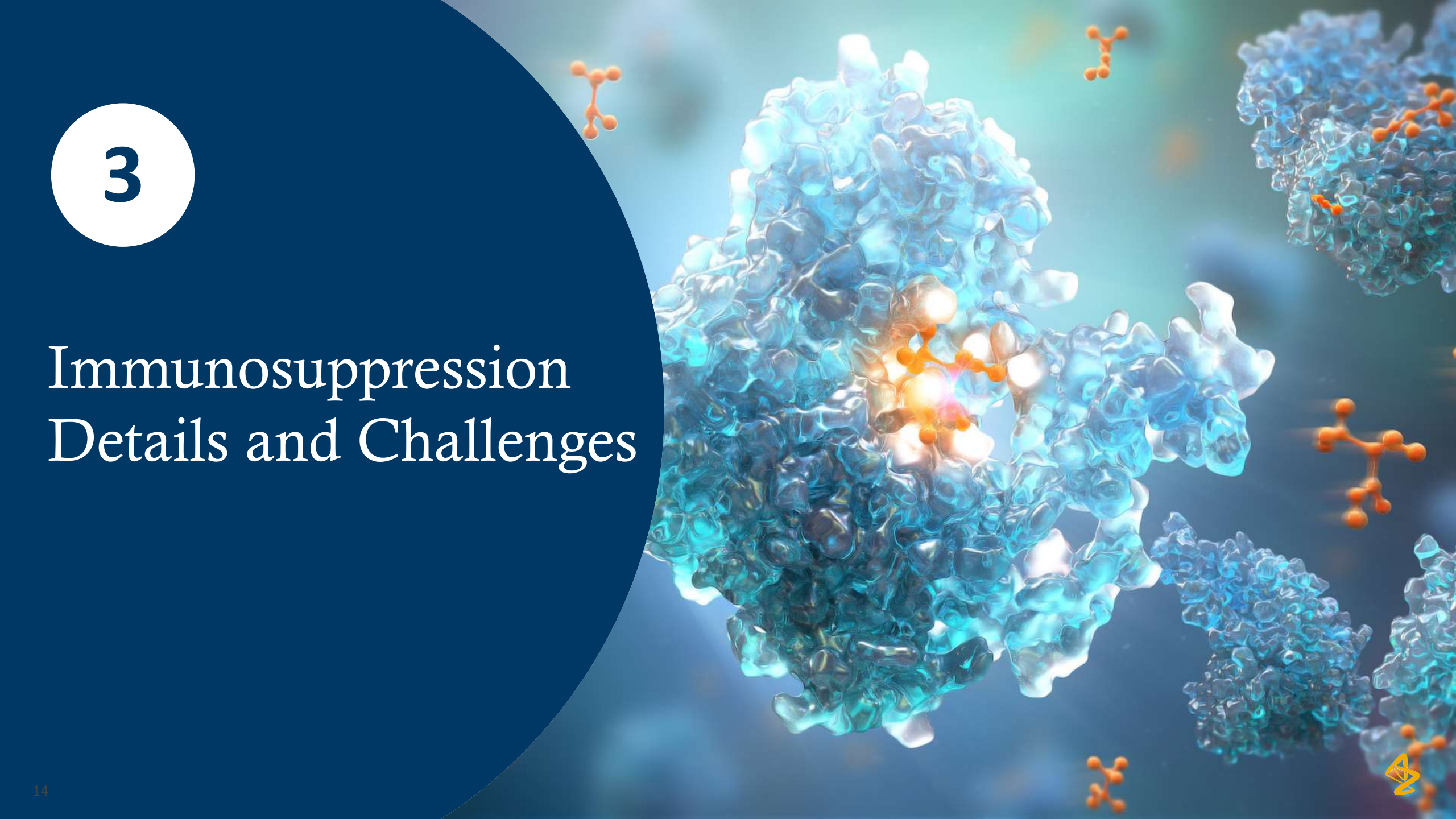


Cardiac Troponin T (CM) Human Nucleoli (HVP derived CM) N-cadherin (cell junctions)

Marcella Sini, Frank Seeliger, Magnus Söderberg

3

Immunosuppression Details and Challenges



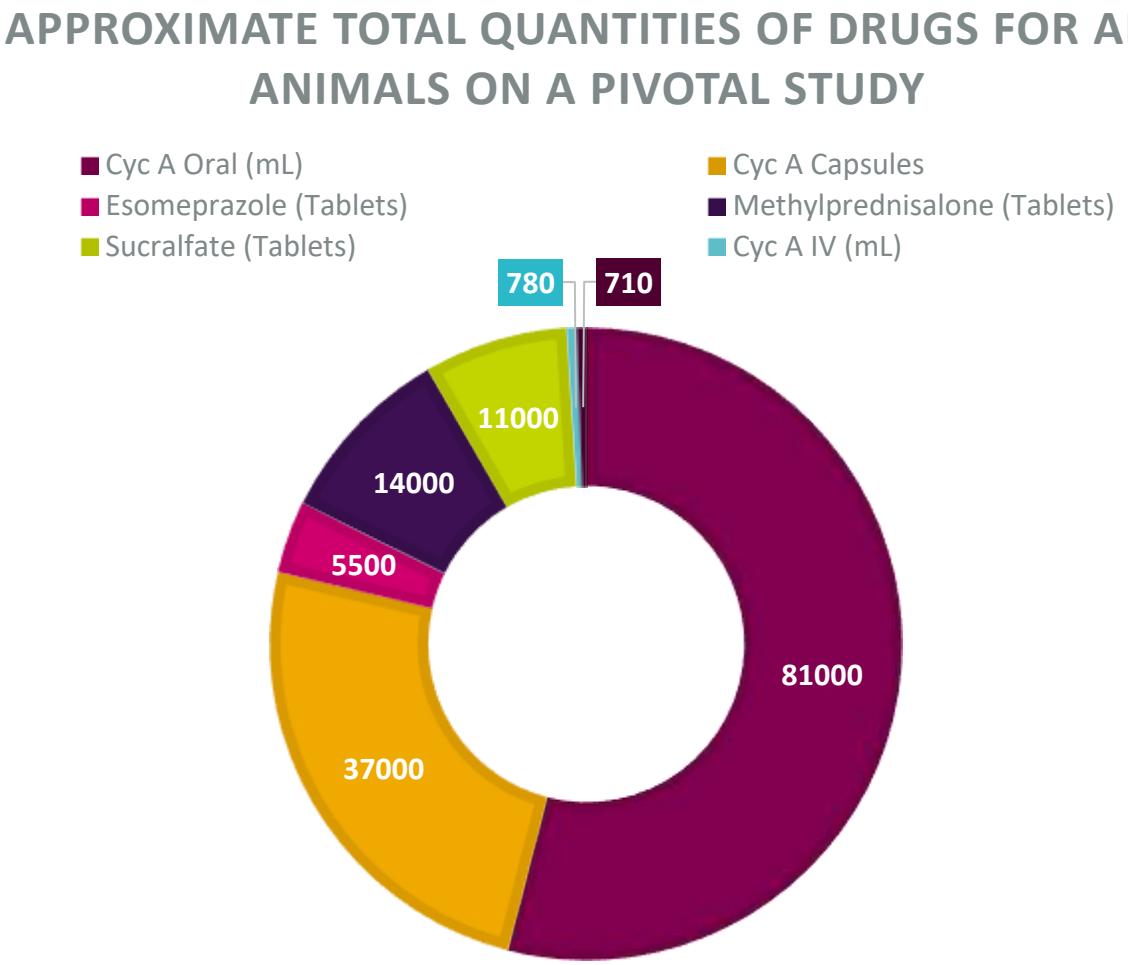
Immunosuppressive regimes and importance of monitoring the levels of IS drugs throughout the study

- Poor or low exposure of the IS drugs can result in poor quality engraftment of the cells at the injection site
- Need to understand the optimal level of exposure:
  - Use of literature for benchmarking
  - Learnings from previous to refine and adjust regime if required
- Establish a dosing regime through PK work or by referring to literature
- Monitor the level of exposure throughout and plan for expedited analysis of samples
- Prepare for adjustments of the regime based on the exposure data being received from the study

Immunosuppresion and Co-medication regime

Establish immunosuppression and co-medication protocols (literature and clinical protocols) that are appropriate for the objectives of the study and relevant to the species. Below is an example of the total quantities of each of the other IS/Co-medication per animal on chronic phase of a study:

- Cyclosporin A
- Methylprednisolone
- Esomeprazole
- Sucralfate
- Cyclosporin A
- Abatacept



Immunosuppresion and Co-medication regime challenges and considerations

- Administration of high numbers of tablets is most challenging and requires a good balance of practical/scientific understanding with animal welfare.
  - Training of animals with limited time frames
  - Restraining animals that have undergone surgery
  - Working with multiple teams in developing appropriate dosing regimens
  - Adaptive/flexible techniques
- Expedited analysis of samples and quick responses to undesired exposure levels
- Many teams are required to obtain large amounts of co-medication for these types of studies.
- Budget - costs of the IS drugs can be high

4

Summary

Points to consider

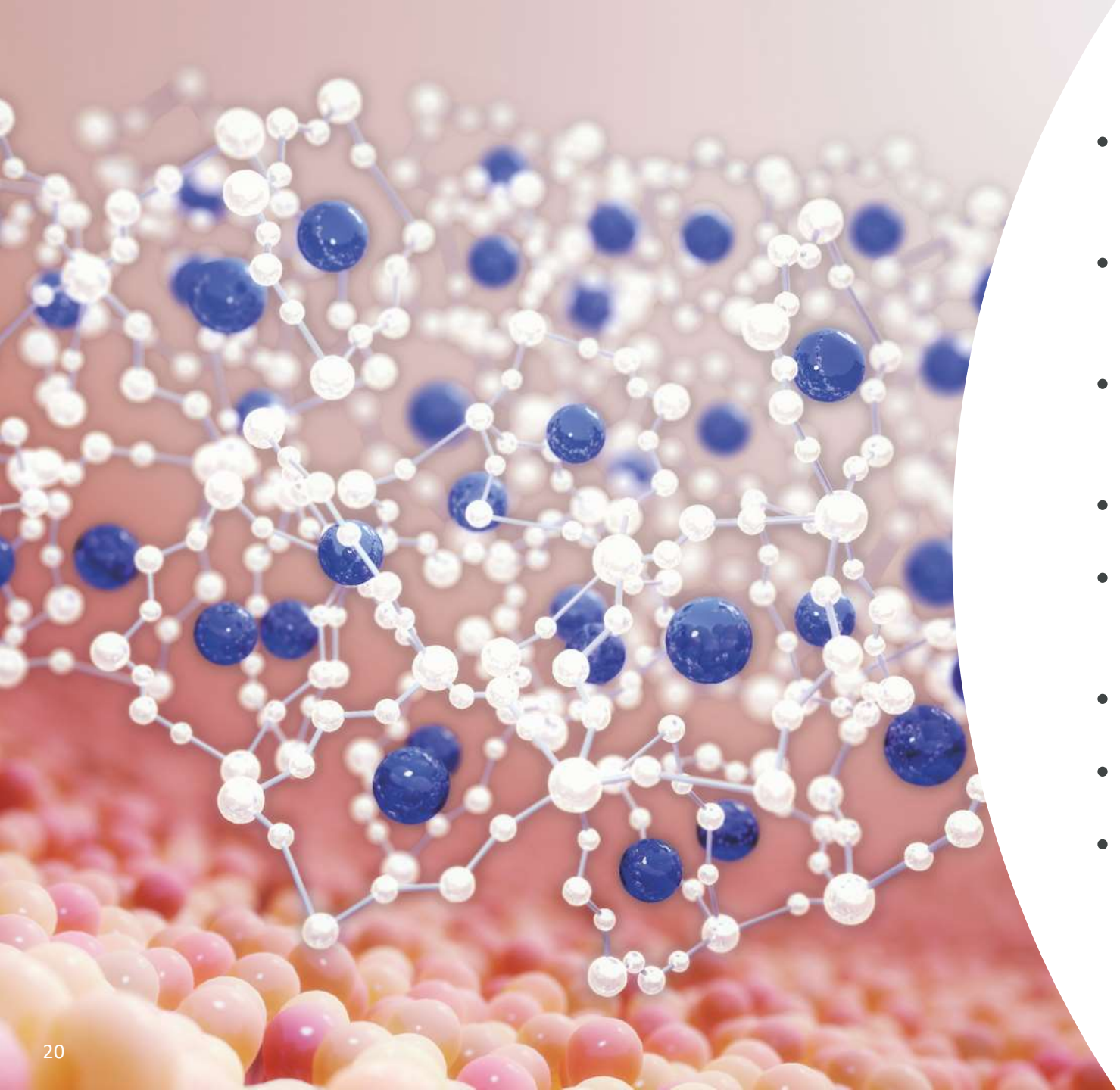
The minipig model presents significant study design and practical challenges that need addressing to ensure successful xenogeneic engraftment following intra cardiac dosing in minipigs.

The implementation of an appropriate immunosuppression regimen is essential for successful engraftment of cells

It is important to confirm procurement of co-medications, navigating the dosing of multiple drugs and monitoring/maintenance of exposure of the comedication.

Supporting animals with appropriate welfare practices is essential in completing the study with good quality data

Costs can be significant and needs to be carefully considered



Acknowledgements

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- Marcella Sini, Associate Director, Tissue Imaging Sciences
- Frank Seeliger, Pathologist
- Magnus Söderberg, Senior Director, Cardiovascular Renal Metabolism Pathology
- Charles River Laboratories, Mattawan
- Procella Therapeutics
- References: https://www.nature.com/articles/s41556-022-00899-8

Heart Ventricular Progenitor Cell Therapy and Using Gottingen Minipigs for Development of Curative Cardiovascular Cell Therapies

David Henry – Associate Director Study Manger, AstraZeneca, United Kingdom

This presentation will provide an overview of the use of cells in curative treatments (highlighting the use of Heart Ventricular Progenitor Cells in heart failure) and considerations for monitoring outsourced studies supporting first time in human (FTIH) trials. This nonclinical model presents significant study design and practical challenges that need addressing to ensure successful xenogeneic engraftment following intra cardiac dosing in minipigs. In addition, the implementation of an appropriate immunosuppression regimen is essential for successful engraftment of cells, for which it is important to confirm procurement of co-medications, navigating the dosing of multiple drugs and monitoring/maintenance of exposure of the comedication. Therefore, an overview of study designs, immunosuppression, the challenges of intra-cardiac dosing cells and supporting animals with appropriate welfare practices will be discussed.

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