AstraZeneca
IMED Biotech Unit

# Pre-clinical bioanalytical support for the modified VEGF-A mRNA program (AZD8601)

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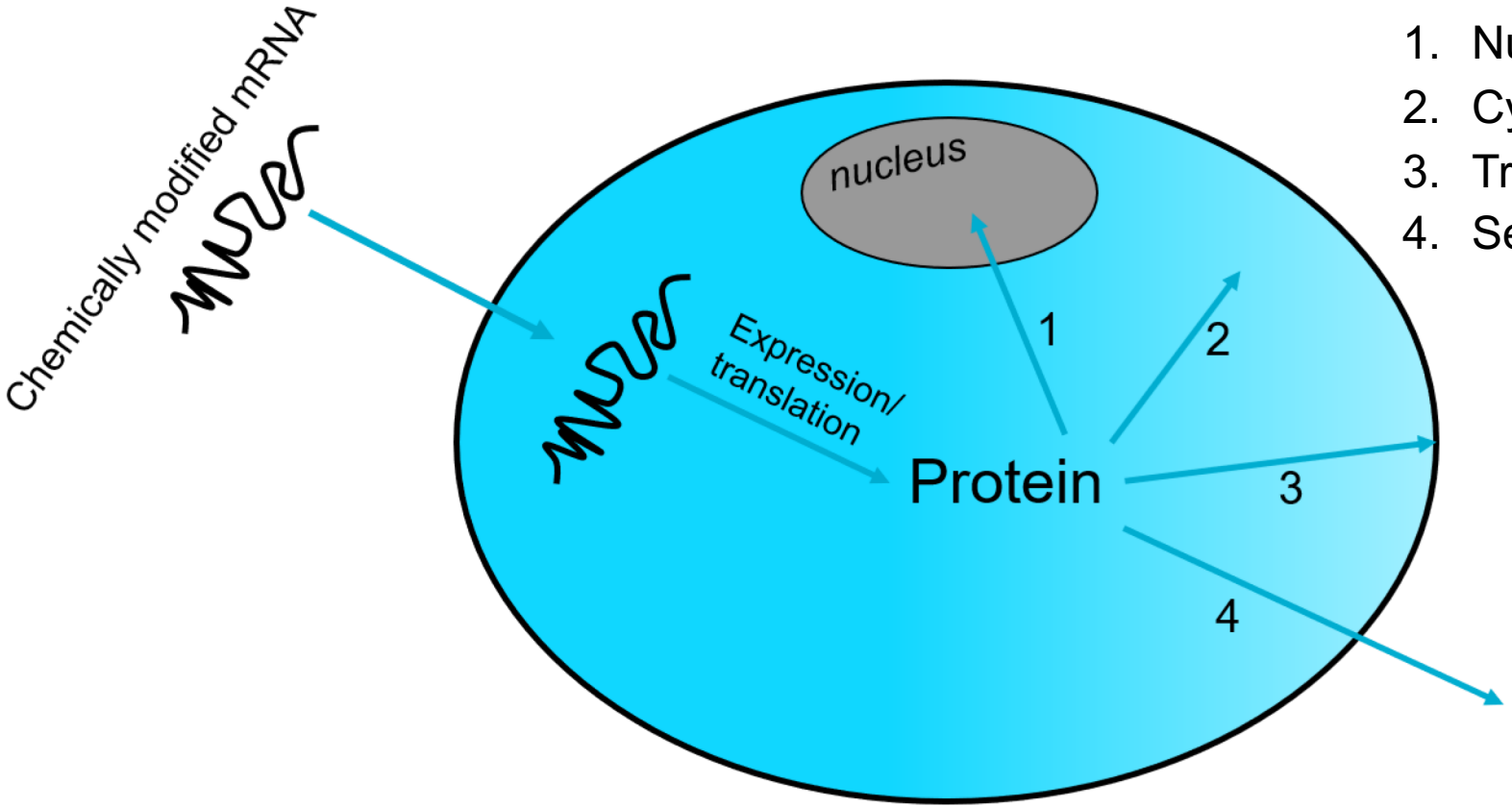
# Pre clinical support for VEGF-A mRNA project (AZD8601)

- What is a mRNA drug?
- Overview of AZD8601 project
- Bioanalytical challenges
- Preclinical enabling work to support clinical program

# mRNA therapeutics overview

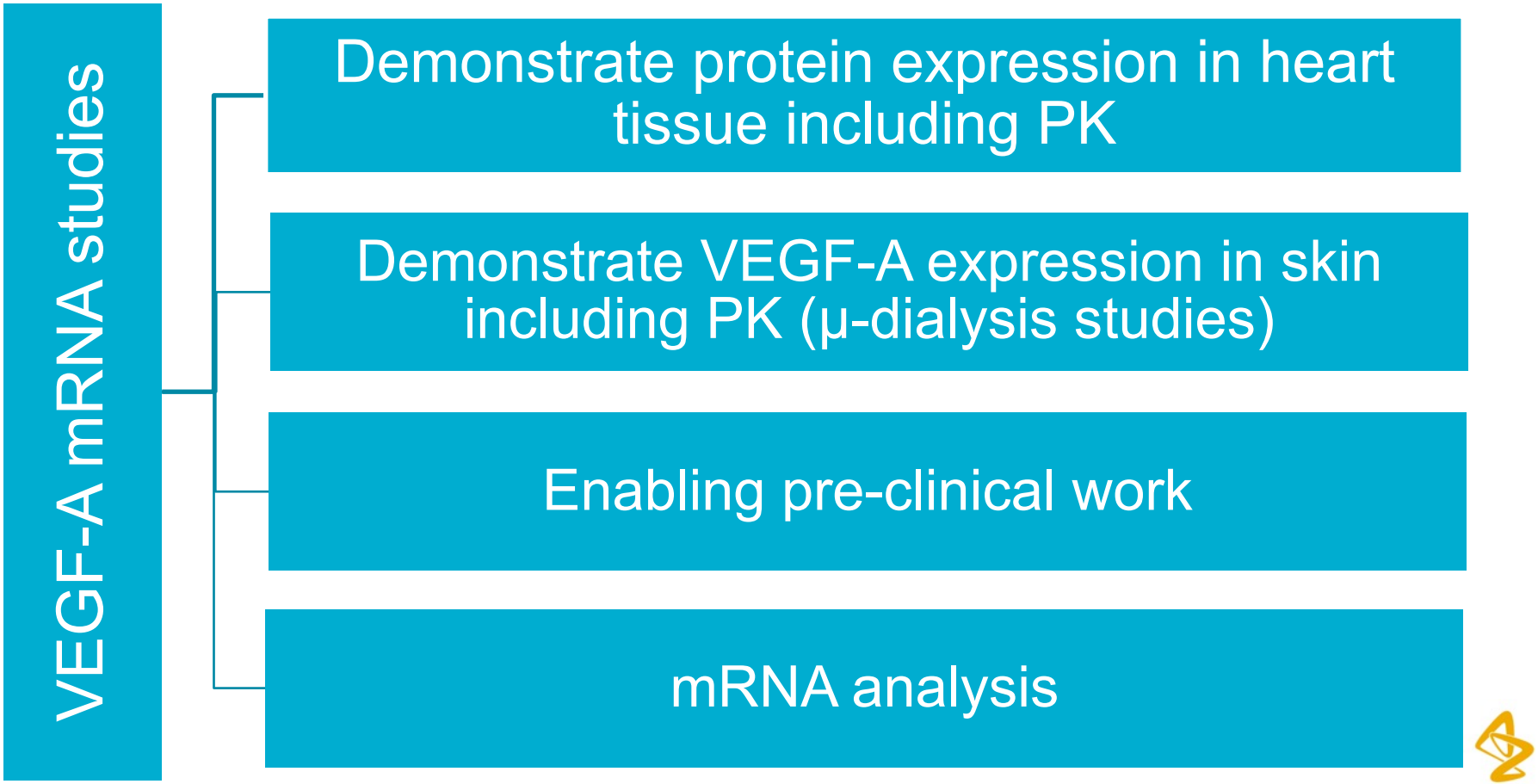



1. Nuclear
2. Cytosolic
3. Transmembrane
4. Secreted

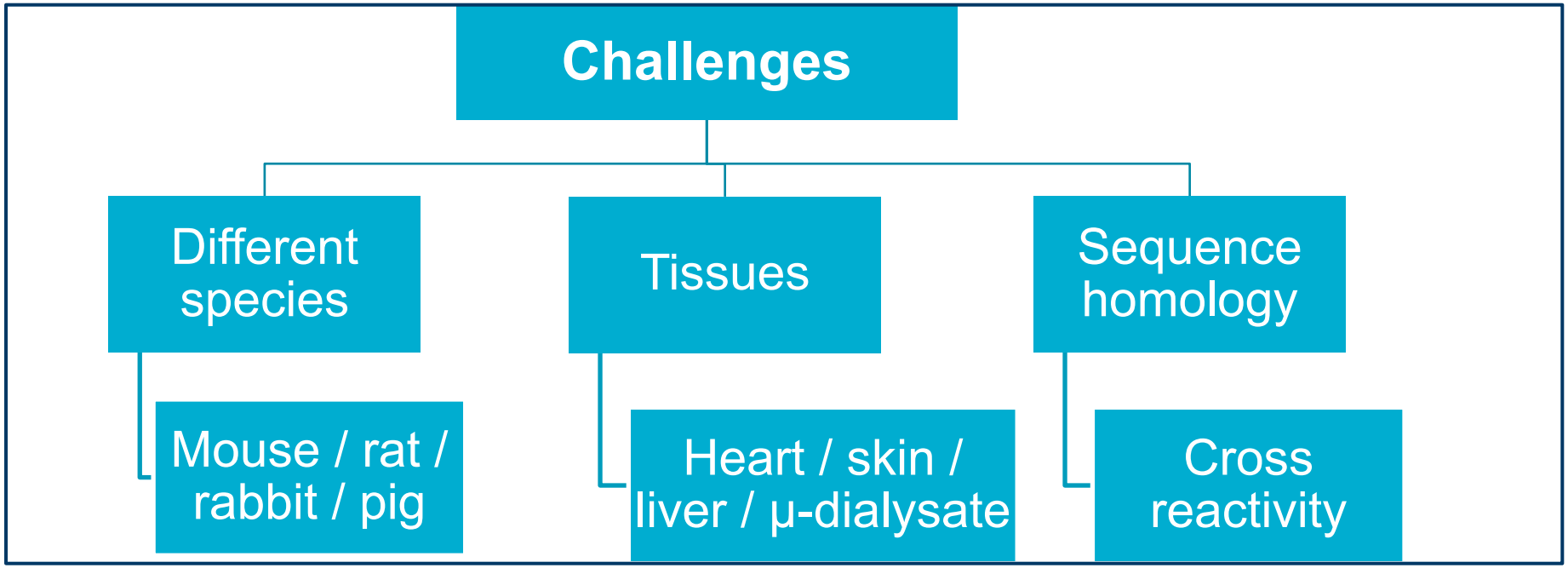
# Overview

| Modified VEGF-A mRNA program (AZD8601) | |
|---|---|
| Background | • Collaboration with Moderna Therapeutics<br>• Cardiovascular, Renal and Metabolism (CVRM) program<br>• The most advanced mRNA program within AZ |
| Target | • Cardiac regeneration / wound healing<br>• Increase blood flow and angiogenesis |
| Project (AZD8601) | • Chemically modified mRNA<br>• Intracardiac / intradermal injection of AZD8601<br>• Citrate/saline solution<br>• Encodes human VEGF-$A_{165}$ protein<br>• Secreted homodimer |

# hVEGF-A mRNA – Pre-clinical study support



# VEGF-A preclinical bioanalytical support




**Solutions**

- Multiple assays
  - MSD-ECL, Gyrolab
- Advanced tissue processing
- Re-evaluation of assays
- Fit for purpose assays

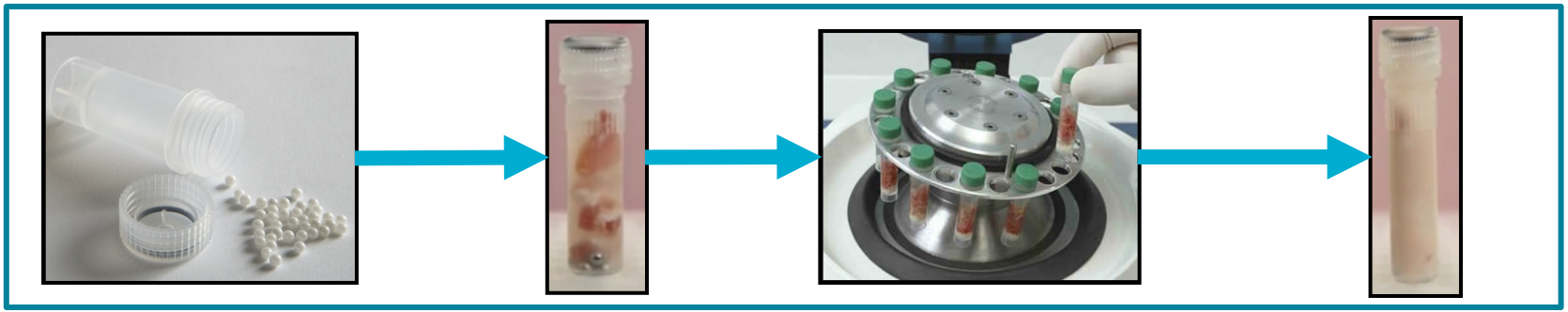
# Intracardiac injections – heart tissue analysis

## Tissues from different species

- 1 mouse = 1 piece
- 1 rat = 5 pieces
- 1 pig = 60 pieces

## Processing

- “High” throughput tissue processing
- Precellys system
  - 5500 rpm
  - Stainless steel beads



# VEGF-A assay for heart tissue analysis

**Assay selection**

- MSD VEGF-A V-plex
- Electrochemiluminescence
- Specific for human VEGF-A
  - Mouse / rat / pig
- LLOQ fit for purpose
- Singlicate analysis

**Cardiac tissue processing**

- Homogenise in MSD Lysis buffer
  - Protease inhibitors
- Compatible with assay
- VEGF-A stable in homogenate
  - 24h at room temperature

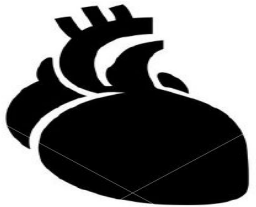
**Adjustments**

- External control
  - Rec. human VEGF-A from WHO
  - Rec. human VEGF-A from R&D

# VEGF-A intracardiac sample analysis

**Intracardiac injection tissue analysis**

- VEGF-A expression in tissue was shown
- PK profile for VEGF-A in rat and pig heart
- Confidence and prediction for clinical studies



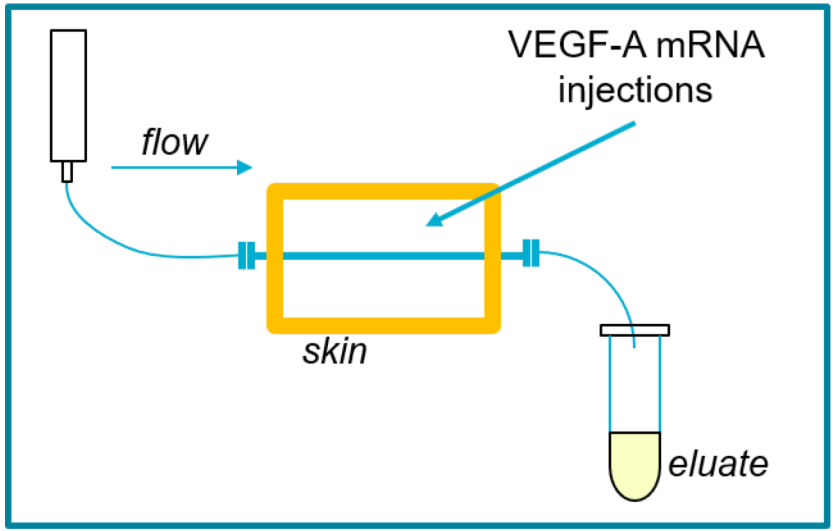
**Continuation**

- Prepare for clinical Phase 1 studies
- µ-dialysis to be used in first studies in human
- Pre-clinical studies to support clinical study
- Test µ-dialysis probes in preclinical setting

# µ-dialysis – enabling work

**VEGF-A in skin µ-dialysate**

- Preclinical work to support clinical program (Phase 1 studies)
- VEGF-A expression in skin after i.d. administration of mRNA
  - µ-dialysis probes
  - µ-dialysis eluate



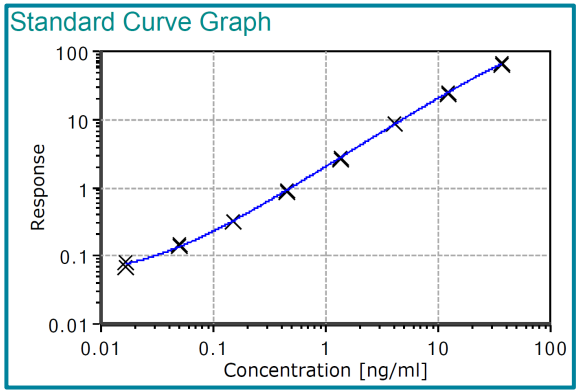

**Rabbit skin**

- Method development
- Proof of concept

# µ-dialysis studies in rabbit – analytical approach

**Assay performance with µ-dialysis eluate**

- Assay previously used unsuitable (interferences)




**Gyrolab assay**

- Develop bespoke Gyrolab assay for VEGF-A
  - Antibodies from R&D
  - Rec. protein from R&D
  - QC protein from WHO
- Diluent 9 from MSD
- 4 µL of sample (1 µL analysed)
- Fit for purpose
- LLOQ: 33.4 pg/mL

Expression in rabbit skin was succesfully shown

# µ-dialysis studies in pigs

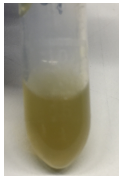
**µ-dialysis in pigs**

- More similar to human skin
- Same µ-dialysis probe as in clinical studies
- Three dose levels of mRNA (24, 120, 600 µg)
  - Intradermal injections
- Gyros and/or MSD VEGF-A assay
  - Adjusted buffers and dilutions
  - LLOQ: 15.6 pg/mL

# Pig skin tissue processing for human VEGF-A extraction

**Pig skin**

- Relatively thick
- No protocols for pig skin processing for protein extraction
- Does not homogenise with standard approaches
- Small molecule extraction method not suitable



**Approach**

- Establish a homogenisation protocol for pig skin
- Balance between skin softening and protein stability
- Avoid formation of gelatine

# Results µ-dialysis vs skin exposure

**Pig skin analysis**

- Similar dose dependency in µ-dialysate and pig skin (at 7.5h)
- Pig skin seems to be slightly more sensitive for demonstrating protein production

# Concluding remarks

- Demonstrated VEGF-A protein expression in target tissue
- Demonstrated VEGF-A protein expression in pig skin tissue
- First in Human in January 2017
- Currently in Phase 2 in CABG patients
    - ClinicalTrials.gov (NCT03370887)

# Acknowledgement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## Moderna Therapeutics


## Confidentiality Notice