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Leaders in Cancer Gene Therapy

How to Approach the Development and Validation to Measure the Expression of Transgene Product

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Outline

- Why do we measure transgene?
- Assay development and validation considerations
- Case Study – NG-350A anti-CD40 mAb transgene
  - Case Study: Development/Fit For Purpose Validation of an Assay to Measure Transgene mRNA in Tumour Biopsies

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Why do we measure transgene product?

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Why do we need to measure transgene product?

Transgene Product

Safety

- Off target events
- Immunogenicity

Efficacy

- Dose Selection
- Bioavailability
- Transient or persistent expression
- Activity
- Proof of mechanism

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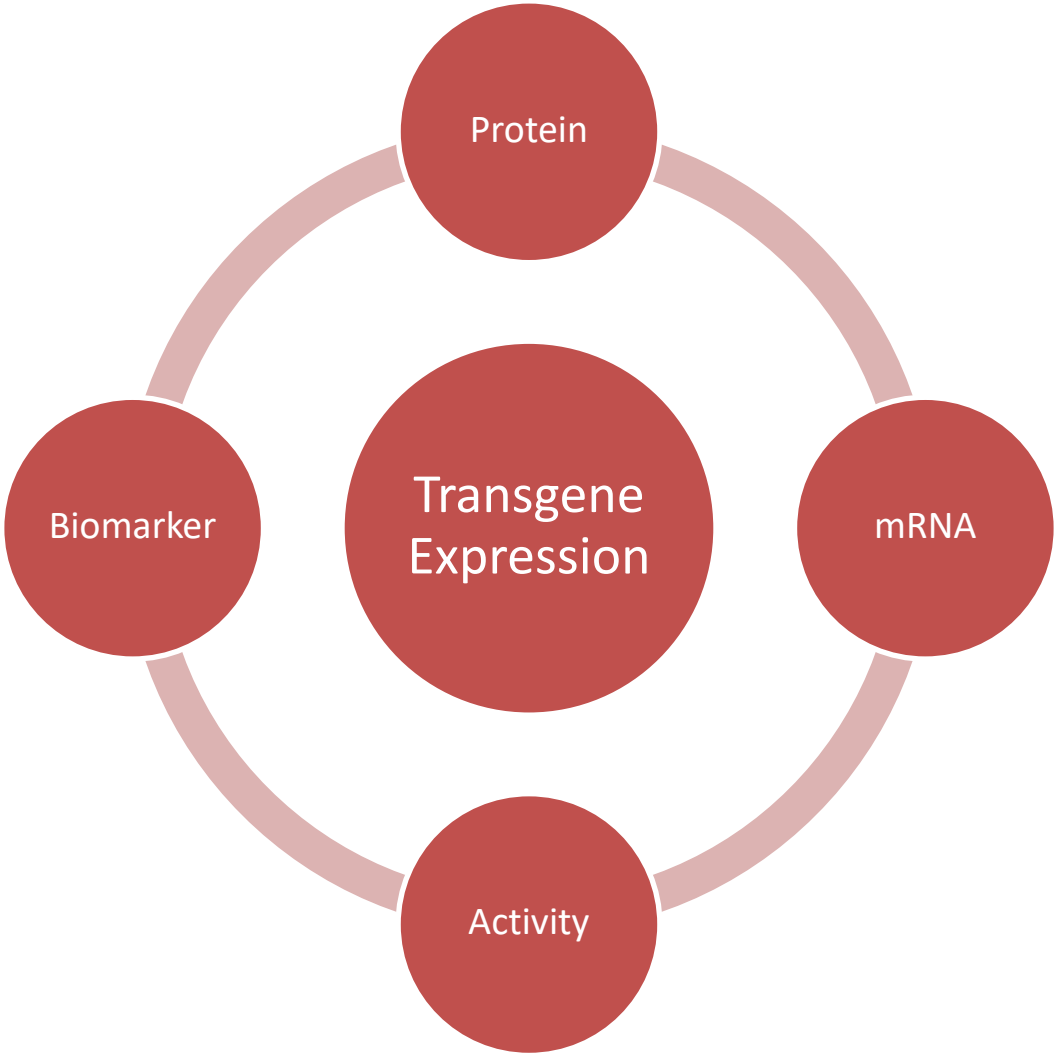
How can we measure transgene product?



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Assay Development and validation considerations

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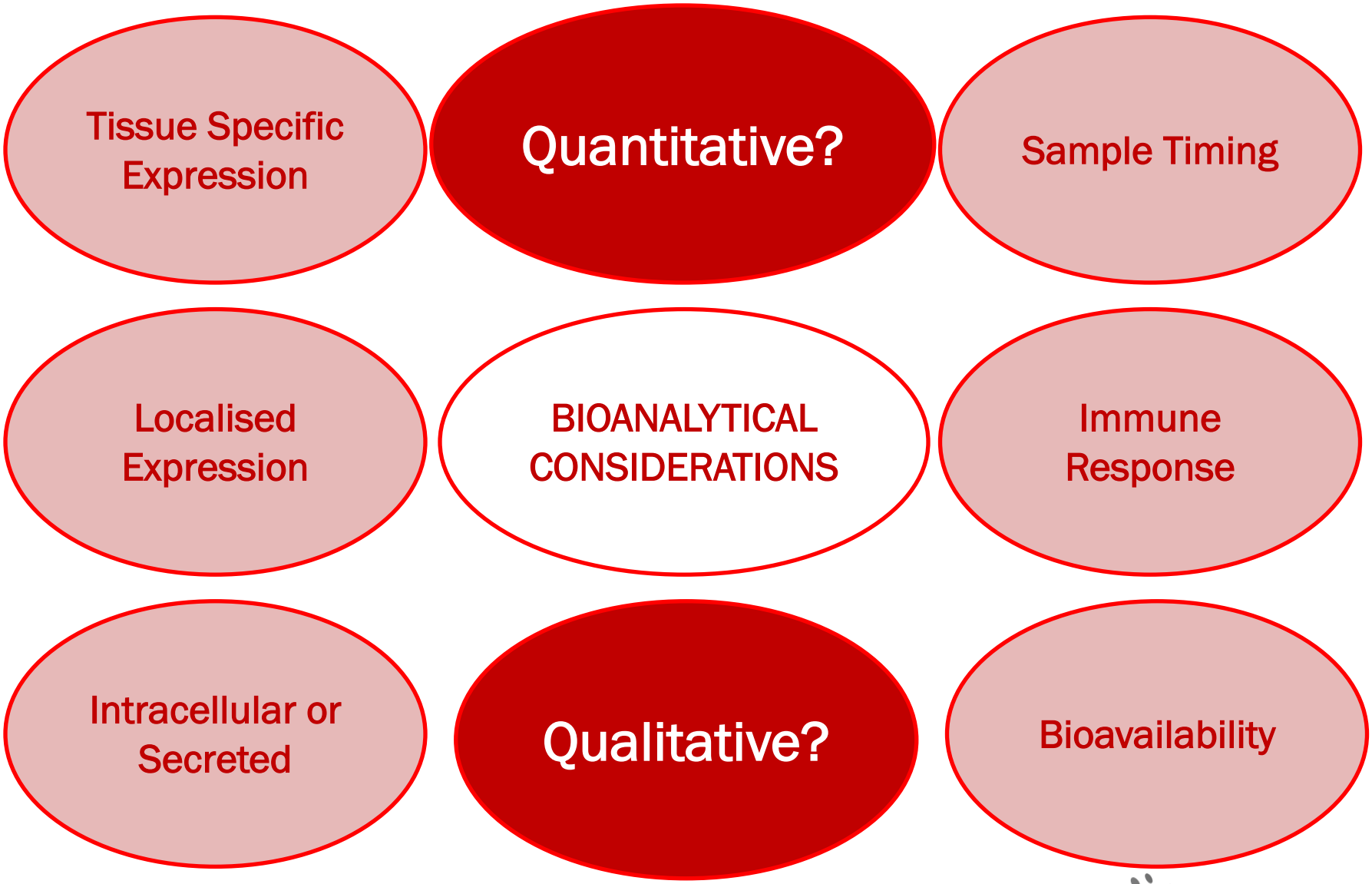
Transgene Expression – Considerations?



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Transgene Assay Validation

Bioanalytical Method Validation

Guidance for Industry

U.S. Department of Health and Human Services
Food and Drug Administration
Center for Drug Evaluation and Research (CDER)
Center for Veterinary Medicine (CVM)

May 2018
Biopharmaceutics

The fit-for-purpose (FFP) concept states that the level of validation should be appropriate for the intended purpose of the study.

Exploratory methods that would not be used to support regulatory decision making (e.g., candidate selection) may not require such stringent validation.

This FFP concept applies to drugs, their metabolites, and biomarkers.

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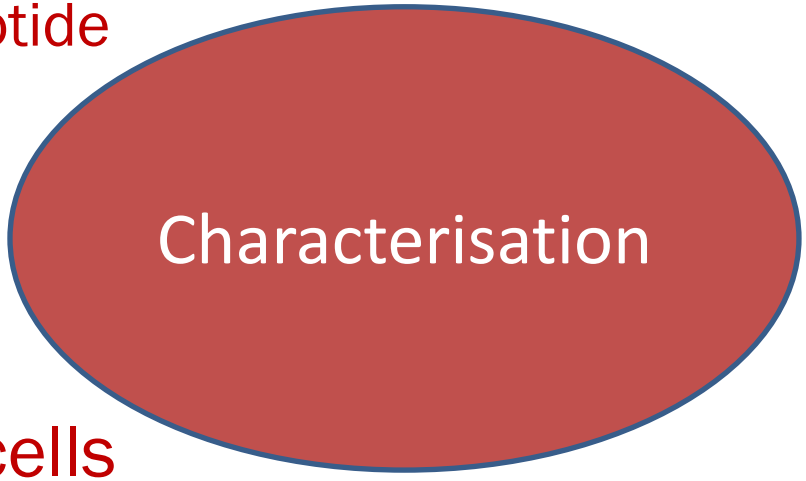
Sample Matrix Considerations

- What is the sample matrix?
  - Blood/Plasma/Serum
    - Normal/Disease state
  - Tissue biopsy
    - Fresh Frozen
    - FFPE
  - Other
- How much sample will be available?
- Is the control matrix available?
- Consider surrogate matrices

Reference Materials Considerations

- What are the reference materials?
  - Protein
    - Peptides
  - RNA
    - Synthetic oligonucleotide
    - Cell extract
  - DNA
    - Plasmid
  - Transfected/infected cells
    - Cell lines/xenografts
    - *Ex vivo* tissues



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Case Study: NG-350A

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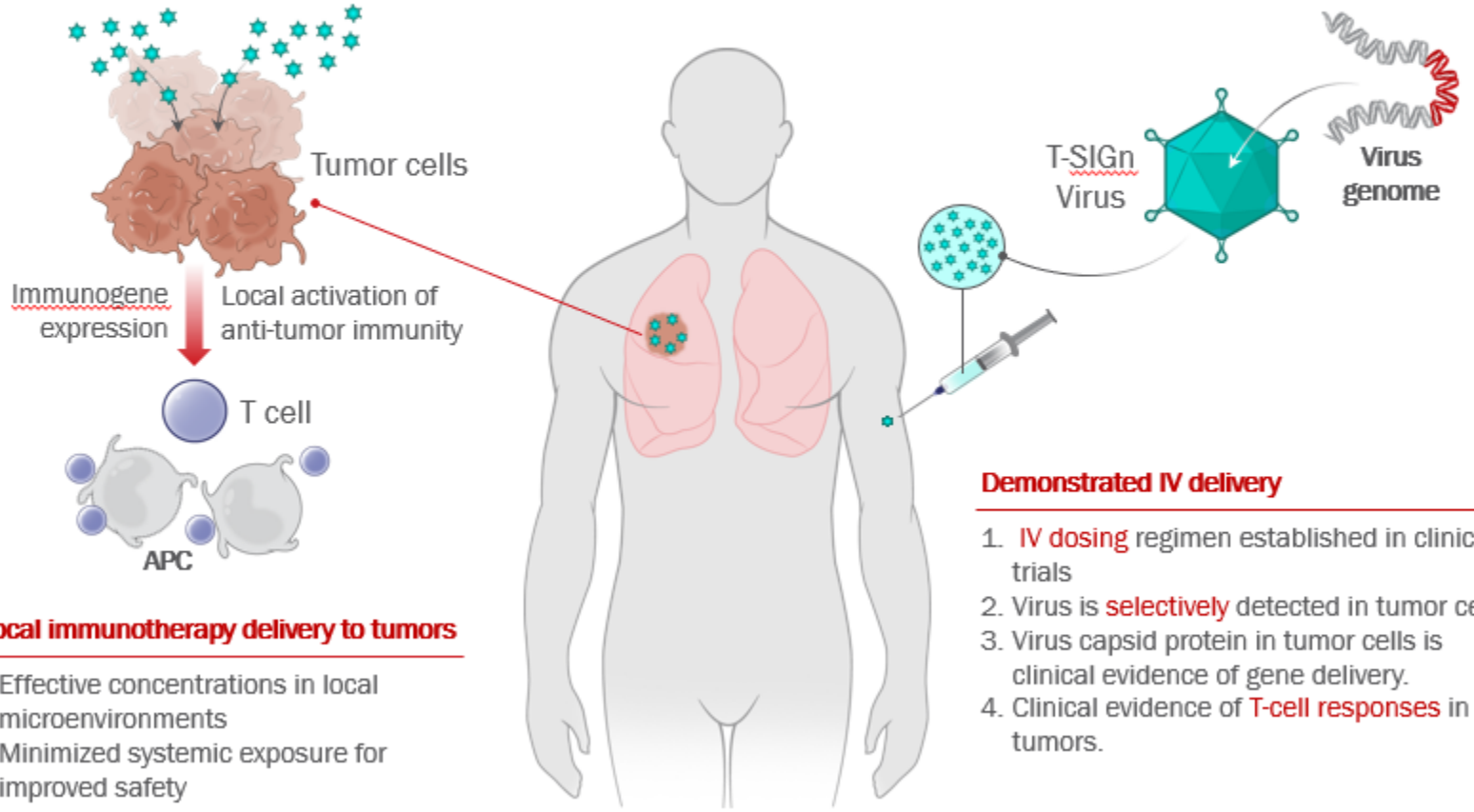
Tumour Specific Immuno Gene Therapy: T-SIGn

Armed enadenotucirev, delivers IV immuno-therapeutic gene therapy to local tumor sites



Local immunotherapy delivery to tumors

- Effective concentrations in local microenvironments
- Minimized systemic exposure for improved safety

Demonstrated IV delivery

1. IV dosing regimen established in clinical trials
2. Virus is selectively detected in tumor cells.
3. Virus capsid protein in tumor cells is clinical evidence of gene delivery.
4. Clinical evidence of T-cell responses in tumors.

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NG-350A Transgene Expression

- What do we want to know?
  - Is the transgene being expressed in the tumour?
  - Is the transgene leaking out into the systemic circulation?

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NG-350A Transgene Analytical Challenges

- Localised tumour expression
  - Needle core biopsies difficult to obtain and small (10 x 1 mm biopsy needle)
  - High sensitivity required
  - Tumour heterogeneity

- mAb binding
  - High affinity
  - CD40 antigen present on B-cells, T-cells, dendritic cells, others?
  - Soluble CD40 in serum

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Assays to Detect Transgene Expression

Assay	Sample Type			
	Tissue biopsy (Fresh Frozen)	Tissue biopsy (FFPE)	Serum	PBMC
Molecular				
➢ RT-qPCR	✓	✓	✓	
➢ NanoString™	✓	✓	✓	
➢ RNAScope ™		✓		
Proteomic				
➢ Mass Spec	✓		✓	✓
➢ ELISA			✓	
➢ IHC		✓		
➢ Flow cytometry				✓

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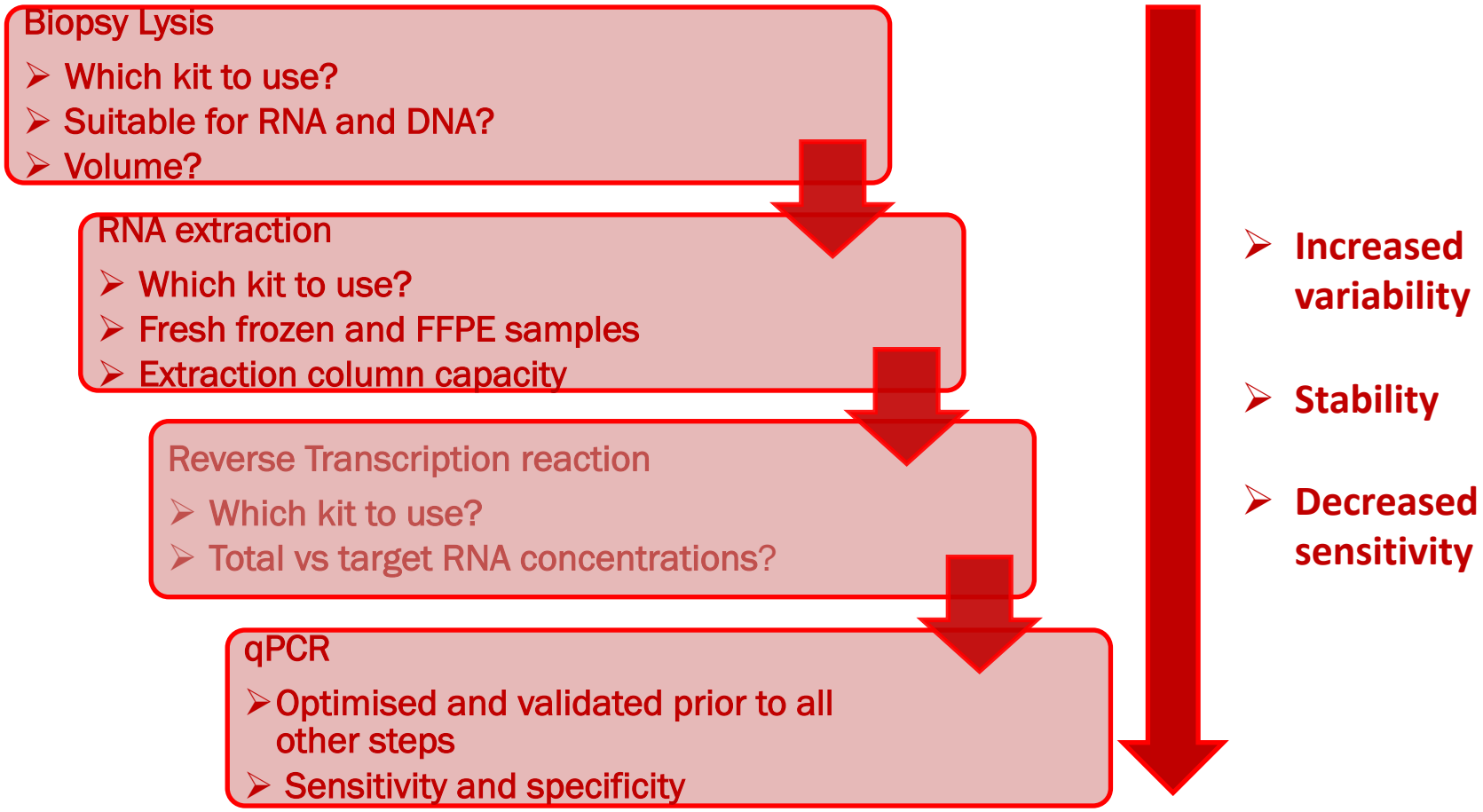
NG-350A RT-qPCR Assay Development: A549 mouse xenografts



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NG-350 Transgene RNA RT-qPCR Validation

- What are the critical assay performance attributes?
  - What is the precision of the extraction procedure?
  - What is the precision of the RT reaction?
  - Does total non-target RNA, and potential contaminants, affect the performance of the RT reaction?
  - Sensitivity – number of infected cells
  - Sample stability
    - Lysate
    - Extracted RNA

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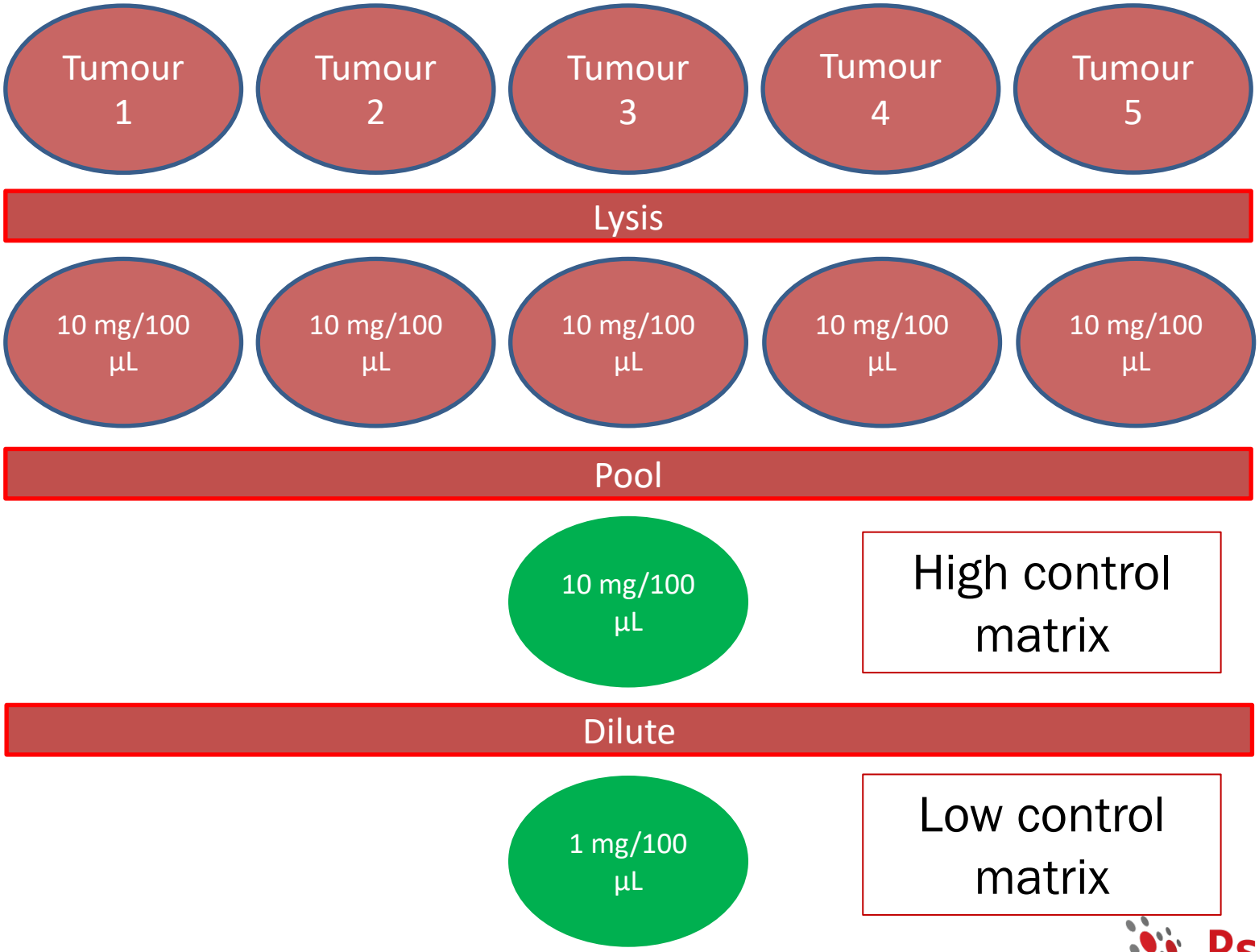
NG-350A RT-qPCR Validation: Control Matrix



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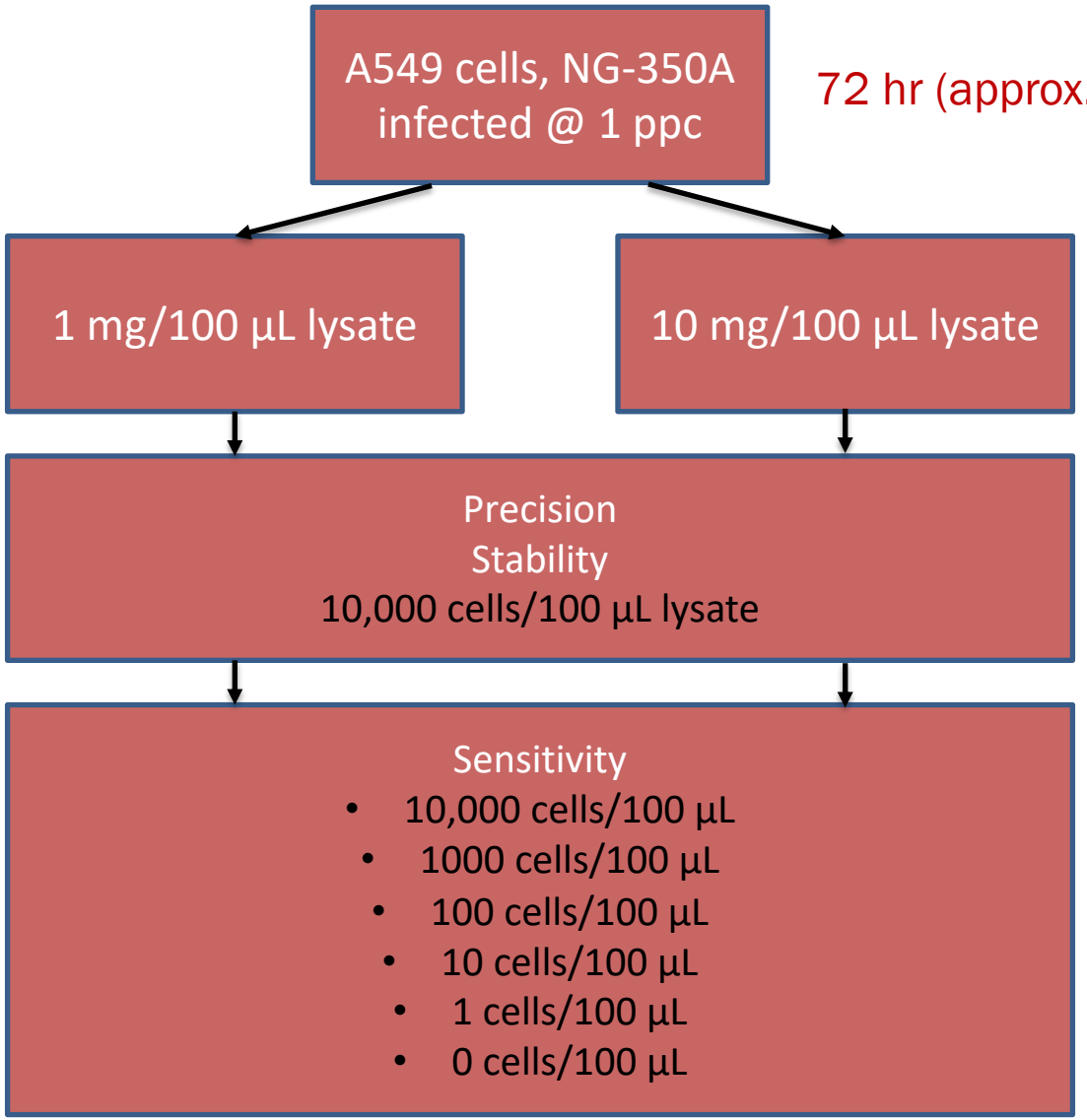
NG-350A RT-qPCR Validation: A549 cell RNA positive control preparation



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NG-350A RT-qPCR Validation: Extraction Precision

Extraction Run	Analyst	1 mg/100 µL		10 mg/100 µL		n
		[RNA] (ng/µL)	Precision (%CV)	[RNA] (ng/µL)	Precision (%CV)	
ER01	1	36.4	6.9	106.9	18.7	10
ER02	2	25.4	7.4	177.0	7.0	6
ER03	1	35.1	12.8	131.4	34.7	4

Observations:

- Extraction precision based on total RNA concentration considered acceptable
- Amount of RNA recovered not proportional to amount of tissue extracted
- Clinical sample RNA range: <5 – 1000 ng/µL

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NG-350A RT-qPCR Validation: RT Reaction Precision - Summary

Lysate conc (mg/µL)	Mean C_T	Mean 350A conc (vp/µL)	Intra-assay Precision (%CV)	Inter-assay Precision (%CV)
1	11.932	6.53E7	≤21.7	21.5
10	14.861	7.84E6	≤11.5	17.9

Observations:

- Intra and inter-assay precision of RT reaction acceptable
  - RT control introduced to monitor RT performance
- Approx 1-log less 350A measured in 10 mg/100 µL lysate than 1 mg/100 µL lysate
  - Suggests inhibition of RT reaction at high total RNA concentrations
  - Samples with high total RNA analysed neat and following dilution

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NG-350A RT-qPCR Validation: Sensitivity

Number of cells spiked	1 mg/100 µL		10 mg/100 µL	
	C_T	[350A] (vp/µL)	C_T	[350A] (vp/µL)
10,000	12.869	4.72E7	14.066	1.25E7
1000	15.861	7.10E6	16.751	2.14E6
100	19.156	8.84E5	19.812	2.81E5
10	21.696	1.77E5	22.762	4.00E4
1	23.862	4.52E4	NR	na
0	UND	na	UND	na

Observations:

- Lower concentrations measured at 10 mg/100 µL
- Number of copies @ 1 cell/100 µL spike higher than anticipated

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NG-350A RT-qPCR Validation: Stability

Stability Condition	Lysate				Extracted RNA			
	1 mg/100 µL		10 mg/100 µL		1 mg/100 µL		10 mg/100 µL	
	[350A] (vp/µL)	% diff	[350A] (vp/µL)	% diff	[350A] (vp/µL)	% diff	[350A] (vp/µL)	% diff
Baseline	4.21E4	-	9.38E3	-	7.60E3	-	7.60E3	-
24 hr Room Temperature	3.11E4	-26.1	8.07E3	-14.0	9.24E3	21.5	9.24E3	21.5
24 hr refrigerated	3.70E4	-12.2	1.51E4	60.9	8.85E3	16.5	8.85E3	16.5
3 additional freeze/thaw	5.13E4	21.9	1.12E4	19.0	6.29E3	-17.3	6.29E3	-17.3

Observations:

- Extracts stable in all conditions tested
- Possible instability of RNA in high concentration lysate following 24 hr in fridge

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Summary

- Little regulatory guidance on requirement to measure transgene expression
  - No guidance on the level of validation required
- Design assay based on samples and questions to be addressed
  - Sample availability
  - Quantitative/Quasi-quantitative/Qualitative
- Perform Fit For Purpose validation based on data requirements, reference standard and matrix availability
  - Sensitivity, specificity & selectivity often key parameters

THANK YOU!

QUESTIONS?

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