Advancing Gene Editing Therapeutics: Pivotal Assessment of mRNA Analytics for Phase 1 to Commercialization

Jeffrey Marshall PhD, Siddharth Bhoraskar PhD

Analytical Research & Development, Beam Therapeutics, Cambridge, MA

Presentation Overview

Beam Introduction

- Our technology
- Our pipeline

Brief quality by design and critical quality attribute assessment overview, method lifecycle pre-IND to Pivotal

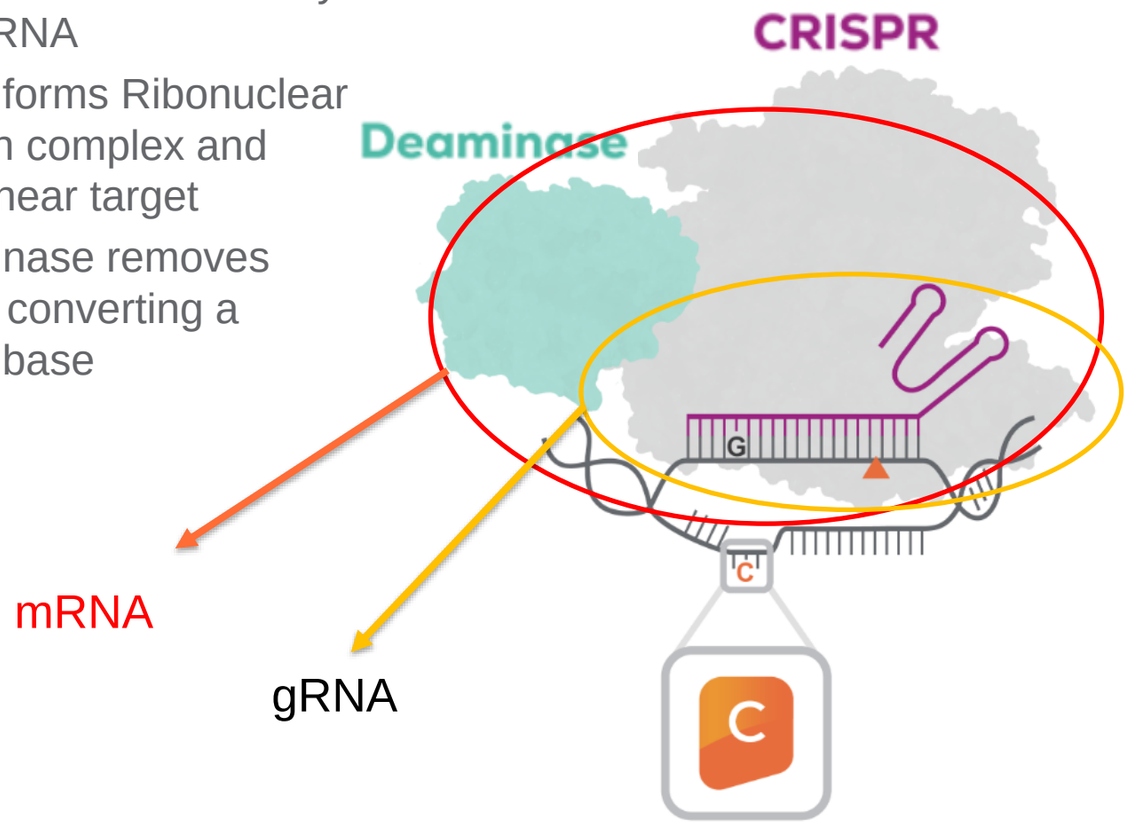
- Quality by Design (QBD)
- mRNA CQAA Examples

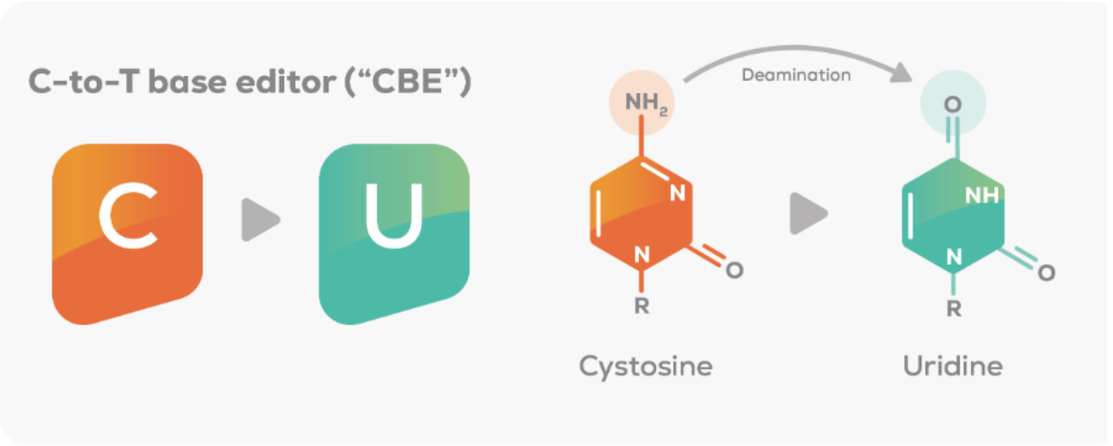
Focused discussion of two mRNA attributes as part of pivotal readiness assessment

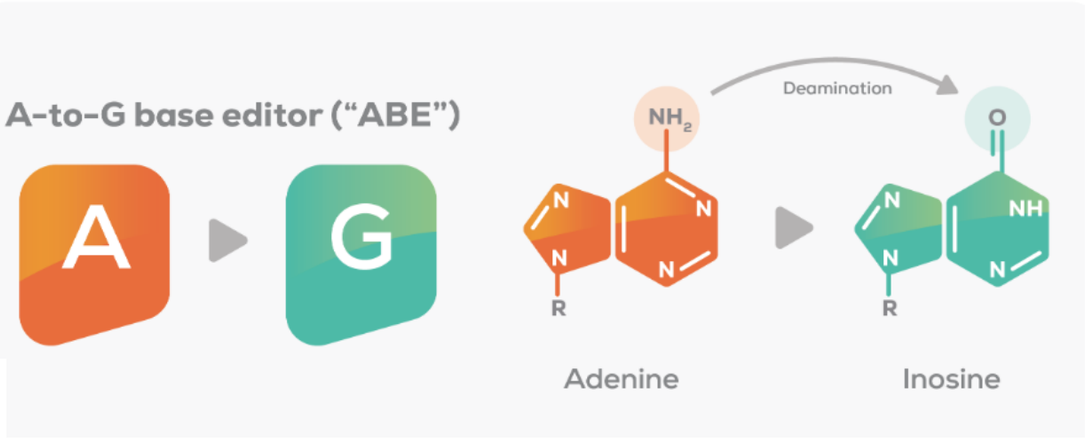
- Poly A tail length and tail heterogeneity
- Covalent base modifications (Oxidation, deamination, depurination, etc.)

Beam Introduction (Our Technology)

- CRISPR-Deaminase Fusion Protein coded by our mRNA
- gRNA forms Ribonuclear Protein complex and binds near target
- Deaminase removes amine converting a single base







Many human genetic diseases are due to point mutations. In fact, amongst the over 50,000 human disease-causing variants described in a mutation database, about 30,000 are point mutations

Beam Introduction
(Where we are 2024-Our Pipeline)

Mix of In-vivo and Ex-vivo programs

PROGRAM / DISEASE		DELIVERY	EDITING APPROACH	RESEARCH	LEAD OPTIMIZATION	IND ENABLING	PHASE I/II	PIVOTAL
BEAM-101	Sickle Cell Disease Beta Thalassemia	*Ex vivo* HSCs	Activation of fetal hemoglobin					
ESCAPE	Sickle Cell Disease Beta Thalassemia	*Ex vivo* HSCs	Multiplex CD117 edit-antibody pair					
BEAM-302	Alpha-1 Antitrypsin Deficiency	*In vivo* LNP	Correction of E342K mutation					
BEAM-301	Glycogen Storage Disease Ia	*In vivo* LNP	Correction of R83C mutation					
BEAM-201	T-ALL / T-LL CD7+ AML	*Ex vivo* T cells	Multiplex silenced CD7 CAR-T					
Complement Pathway (Apellis)		*In vivo* LNP	Undisclosed					
3 undisclosed targets (Pfizer)		*In vivo* LNP	Undisclosed					

Continue to build product and process understanding. Feed back into control strategy

LNP = Lipid Nanoparticle; HSC = Hematopoietic Stem Cell; T-ALL / TLL = T-Cell Acute Lymphoblastic Leukemia / T-Cell Lymphoblastic Lymphoma; AML = Acute Myeloid Leukemia; ESCAPE: Engineered Stem Cell Antibody Paired Evasion

Iterative Process of Quality by Design (QBD) and Critical Quality Attribute Assessment (CQAA)

QBD is an iterative process where product understanding and process understanding feed into each other to build and strengthen the product and process control strategy. QBD is iterative and responds to inputs from structure function studies, pre-clinical studies, clinical data, and process characterization studies (refer to ICH Q8R2).

Abbreviations:
QTPP = Quality Target Product Profile
RRF = Risk Ranking and Filtering
CQA = Critical Quality Attribute
PC = Process Characterization
PV = Process Validation
CPP = Critical Process Parameter

Sarah Demmon, Swapnil Bhargava, Doreen Ciolek, Jennifer Halley, Nomalie Jaya, Marisa K. Joubert, Edward Koepf, Phillip Smith, Melody Trexler-Schmidt, Philip Tsai,A cross-industry forum on benchmarking critical quality attribute identification and linkage to process characterization studies,Biologicals,Volume 67,2020,Pages 9-20,ISSN 1045, 1056,https://doi.org/10.1016/j.biologicals.2020.06.008.

Example of mRNA CQAA and Focus on Today's Presentation

- Snippet of CQAA for mRNA detailing the attribute, safety/efficacy rationale summary, notes/references and data, and risk assessment for safety, efficacy, and uncertainty.
- All three risk assessment parameters (safety, efficacy, and uncertainty) are combined to determine criticality of the attribute.
- Document is living and subject to change/iteration as data becomes available. CQAs and justifications can be communicated to regulatory bodies for late-stage products

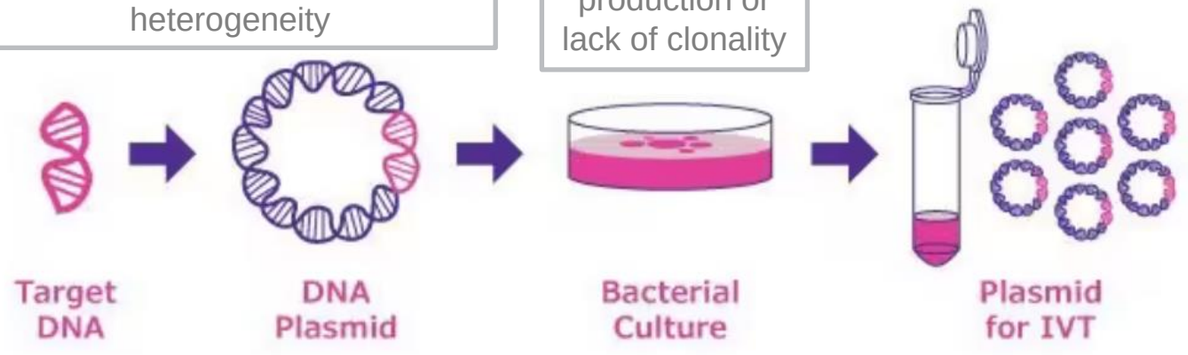
Attribute Class	Attribute	Rationale	Additional Notes and References	Safety	Activity/ Efficacy	Uncertainty
Purity	Tail length and heterogeneity	Safety: unknown impact	Longer tail can increase mRNA stability/half-life. short poly A tail may result in exonuclease attack on 3' end and consequent product degradation. Generate internal data for different tail length impact on potency and safety	L	M	M
		Efficacy: can reduce efficacy				
	Covalent Base Modifications	Safety: Unknown	Oxidation products, depurination, crosslinking can reduce translation efficiency. Deamination will change the base ID and potentially affect the coding sequence. Oxidation, depurination, and deamination are strongly influenced by pH and temperature.	M	M	H
		Efficacy: can reduce efficacy				
	Additional Attributes that can affect safety and efficacy that our outside scope for today…					

Tail Length and Tail Heterogeneity Background

Plasmid DNA to mRNA manufacturing

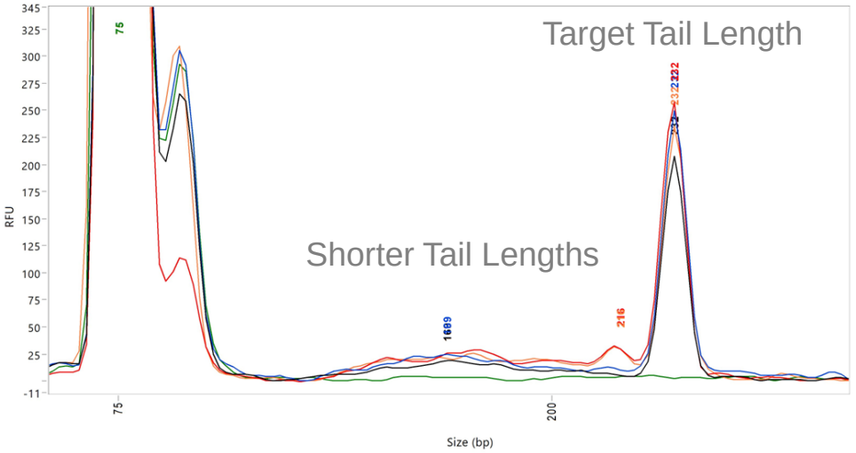
Plasmid design/orientation optimization may reduce heterogeneity

Instability of cell line during production or lack of clonality



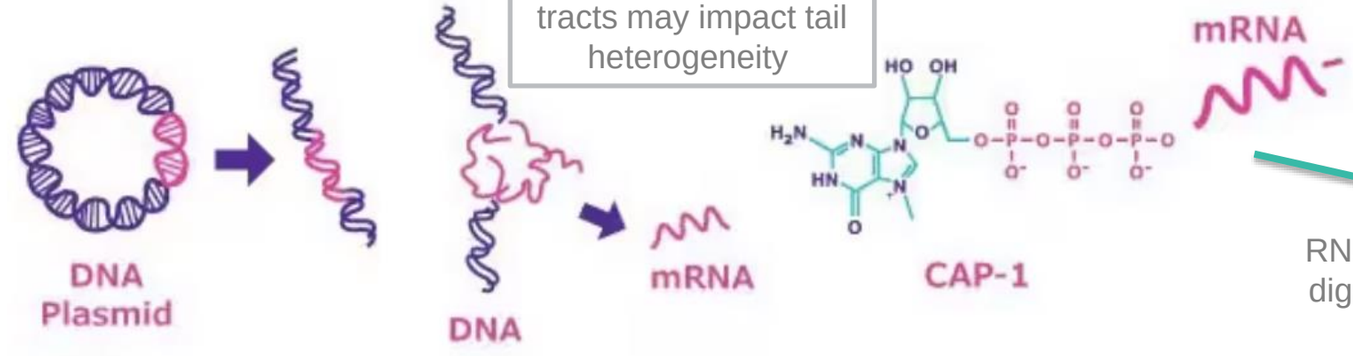
Plasmid digestion

CGE of Plasmid tail digestion product

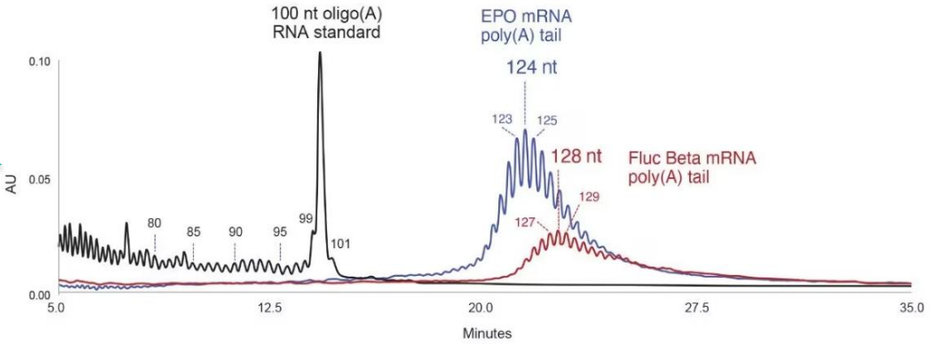


Liquid Chromatography Methods for Analysis of mRNA Poly(A) Tail Length and Heterogeneity
Anal. Chem. 2023, 95, 38, 14308−14316, September 11, 2023
https://doi.org/10.1021/acs.analchem.3c02552

IVT Enzyme infidelity on homopolymer tracts may impact tail heterogeneity



RNaseT1 digestion



Tail Length and Tail Heterogeneity CQAA

Is it a CQA?

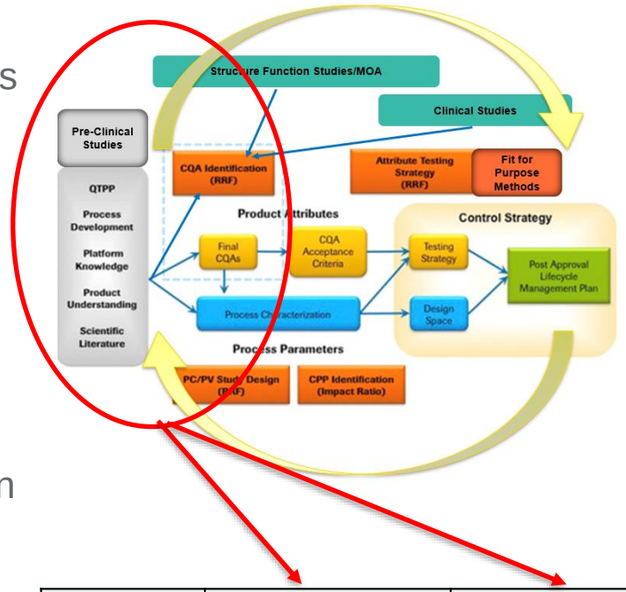
- Literature consensus is that shorter tails on average lead to shorter mRNA half-life. Conclusions are mixed when it comes to translation efficiency **(TE)** except for tails <~30nt where TE drops
  - Provides endonuclease protection as well as poly A binding protein stable loop for translation initiation
  - Cell type play a major roll in mRNA half-life and TE
- ex-vivo vs. in-vivo (LNP) drug products differ dramatically *making it difficult to leverage platform knowledge across modalities*
- Structure-function studies using varied poly A Tail length constructs
  - For ex-vivo: perform mRNA potency/protein expression as well as electroporation of human cells at standard dosing concentration and sub-saturating doses to make more sensitive to potential changes in TE
  - For in-vivo (LNP): perform mRNA potency/protein expression testing as well as LNP DP potency testing to determine effect.

How to measure it?

- pDNA digestion-CGE, pDNA bidirectional sequencing of supercoiled
- mRNA digested IPRP, IEX, SEC, CGE, and ddPCR

How to control for it?

- In the pDNA production release and/or mRNA process release? Build process understanding and relationship with the attribute
- Average length? Distribution? Specifications (>eg.80nt)? Use process characterization and structure function studies to inform control strategy



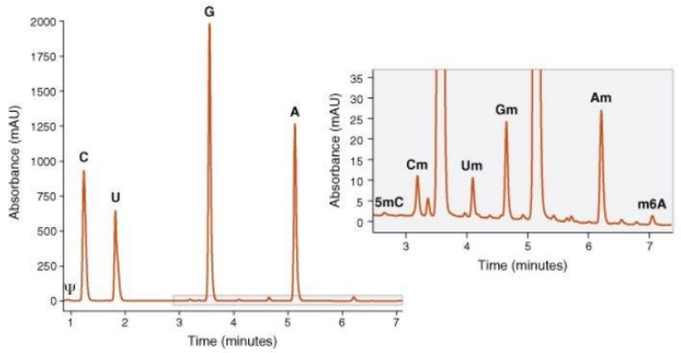
Safety	Activity/ Efficacy	Uncertainty
L	M	M

Is it a QCQ? YES.

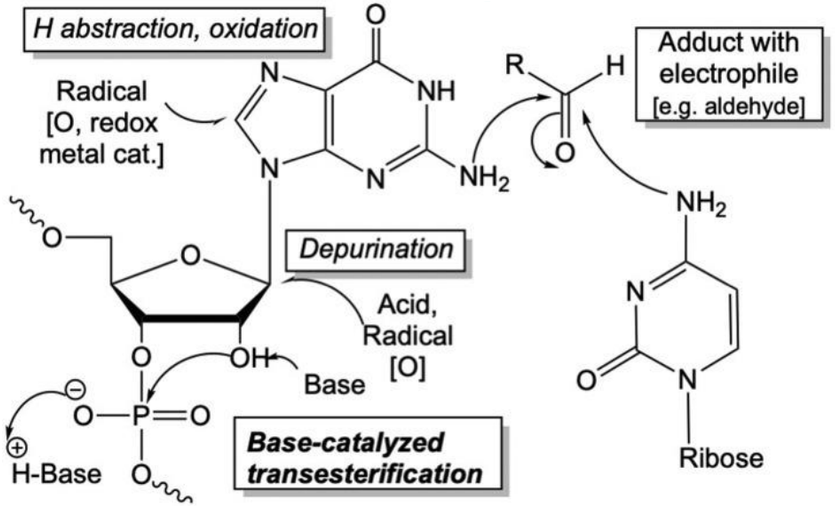
Assessment is revisited as data comes in. Uncertainty should decrease as more information is gained

Covalent Base Modifications Background

- Bases A, G, C, and U can undergo many covalent modifications that impact the fidelity of the mRNA
- “Covalent base modifications” is a general term to encompass several types of chemical changes generated by different mechanisms
  - Oxidization
  - Deamination (in unbuffered water)
  - Depurination
  - Cross-linking or adduct formation
- Rate of formation are affected by pH, dissolved oxygen, metal impurities, and UV.
- Translation efficiency is reduced by interruption of the coding sequence with base modifications (G→8oxG, A-->I, G-->X, etc.) caused by oxidation, deamination (water), oxidative crosslinking, UV degradation products (can include radical crosslinking), etc.



NEB: A Fast One-Step Digestion of DNA or RNA for Global Detection and Characterization of Nucleotide Modifications



Blenke, E. O. *et al.* The storage and in-use stability of mRNA vaccines and therapeutics: Not a cold case. *J. Pharm. Sci.* (2022) doi:10.1016/j.xphs.2022.11.001.

Degradation products	Source
Oxidation of bases	Auto-oxidation, Metal residues, Light
Depurination (abasic site)	Hydrolysis (acid), Oxidation
Deamination-hydrolyzed bases	Hydrolysis (acid)
RNA Fragments	Hydrolysis (base), Heat, Peroxides, H_2O_2 , RNase enzymes

Covalent Base Modifications CQAA

Is it a CQA?

- There are many literature sources detailing the negative effects of base oxidation and side reactions on TE. This potentially can influence efficacy and requires the following considerations:
  - Manufacturing process may impact this attribute class, exposure to metal ions and other catalysts for oxidation. Process ranges and hold times should be evaluated
  - Perform forced degradation (**FD**) studies, generally keep conditions close to process extremes and monitor to inform process characterization studies
  - Utilize the mRNA formulation or in-process matrix as this affects degradation pathways
  - Long term storage conditions and stability studies should also be evaluated
- Structure function studies

  Determine influence on potency and/or protein expression (Rabbit Reticulocyte or target cell based protein expression) using FD material

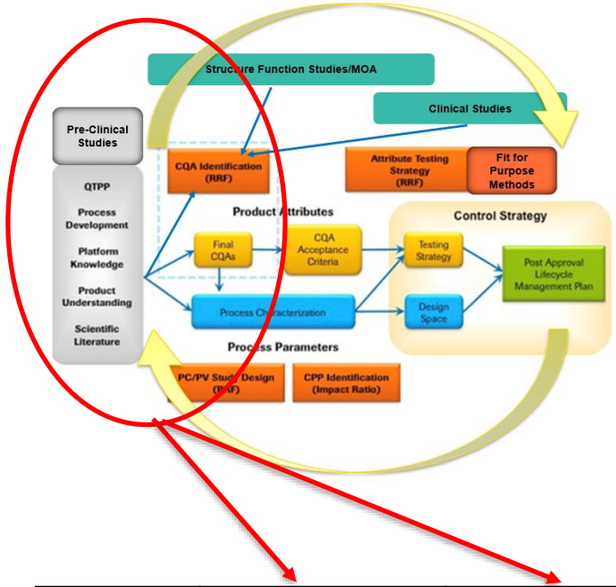
  Correlate % base modification to protein expression

How to measure it?

- RP/IPRP Single nucleotide or nucleoside analysis, Nuclease digested mRNA-LC/MS

How to control for it?

- Initially use structure function, process ranging, and any pre-clinical data to drive control strategy. Influence on potency may lead to testing on release and stability



Safety	Activity/ Efficacy	Uncertainty
M	M	H

Is it a QCQ? YES.

Assessment is revisited as data comes in. Uncertainty should decrease as more information is gained

Summary

- Quality by design concepts can be used to build safer products through iterative cycles of studies focused on process and product understanding.
- In general structure function study parameters should be based on the production process as well as the patient dose administration.
- CQAA are built over time and are integral to building the product control strategy
- Tail length is a CQA affecting TE, however, the degree of which needs to be evaluated for each specific molecule and target system
- Covalent modifications as a class of attributes are also CQAs and can lead to a reduction in TE. Specific forced degradation studies in formulation and in-process conditions should be assessed to determine the risk level