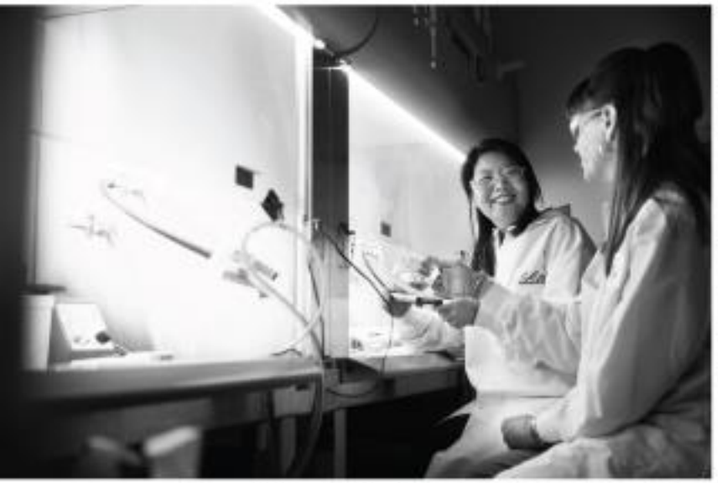
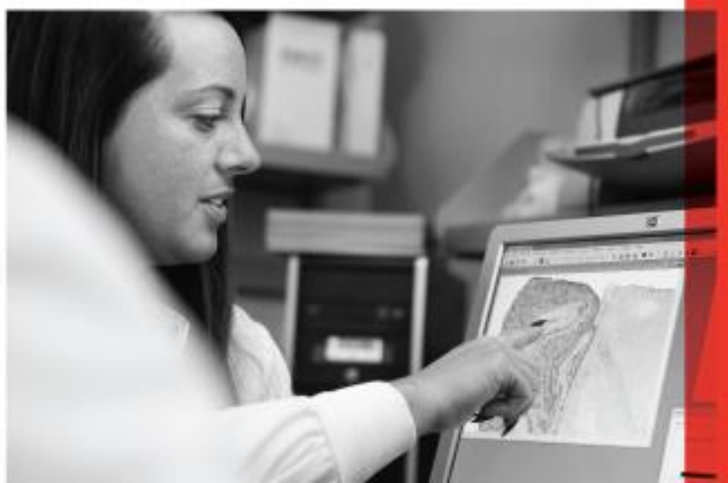




Forced Degradation Studies

in Support of Product Development and Registration

Elisabeth Krug

Outline

- Definitions
- Elements of the Forced Degradation Strategy
- Degradation Pathways
- Stress Testing Studies in Clinical Development
- Formal Stress Stability Studies
- Summary and Conclusion

Definitions

- *Forced Degradation*:
  A forced degradation study is defined as the intentional degradation of the drug substance and/or drug product to an appropriate extent by means of various stress testing utilizing conditions such as pH, temperature, light, oxidizing agents, as well as potential mechanical and chemical stresses that occur as part of the manufacturing/ distribution process.

  Stability and Accelerated Stability testing:
  Studies done in accordance with ICH and regulatory guidelines quiescently at the proposed long-term and elevated temperature conditions

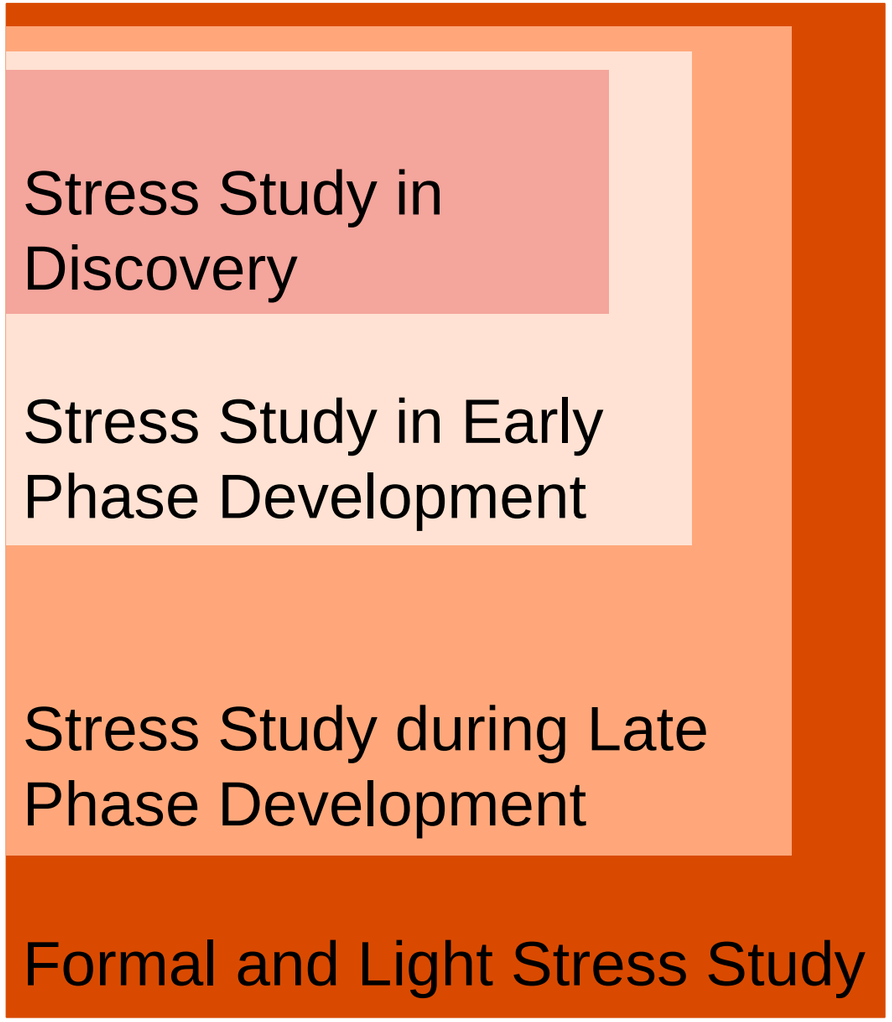
- Forced degradation, including dedicated stress testing, therefore, is an integral part of product development.

Overall Stress Testing Strategy

- “Wherever significant qualitative or quantitative changes indicative of degradation product formation are detected during long-term, accelerated and/or stress stability studies, consideration should be given to potential hazards and to the need for characterization and quantification of degradation products within the long-term stability program...” (ICHQ5C)

- Continuously increase the knowledge about the molecule to ensure that the primary degradation pathways are identified and monitored with appropriate analytical methods during drug substance and drug product process and formulation development as well as during stability.

- Use a phase appropriate approach while ensuring patient safety



Degradation Pathways

Degradation Pathway	Most Susceptible Residues	Conditions Affecting Degradation
Oxidation	Met, Cys, His, Trp, Tyr	Temperature, peroxides, light, metal ions, oxidizable buffer species (such as histidine)
Hydrolysis/Fragmentation	Asp and Protease specific targets	pH, proteases
Deamidation	Asn, Gln	pH, buffer species, ionic strength, temperature
Racemization	His, Asp, Ser	pH (basic)
β-Elimination and Disulfide Exchange	Cys	pH (basic), buffer species, metal ions, temperature, thiol scavengers, oxidizing agents
Physical Instability (e.g., particulates, phase separation)	No specific predictive residues	Temperature, pH, buffer species, ionic strength, cations, agitation, shear, hydrophobic surfaces, lubricants, polymers/elastomeric surfaces, light
Crosslinking	Lys, Ser, Asp, Asn, Gln, Cys	pH, buffer species, light, temperature
Isomerization	Asp	pH, buffer species, ionic strength, temperature
Aggregation	No specific predictive residues	Physical stress – agitation/air-liquid interfaces, heating, freezing and thawing, light
Pyroglutamate formation	N-terminal Gln and Glu	Temperature, pH
Interaction with matrix components	Lys and N-terminal amine (glycation, citrylation)	Matrix components, process conditions

Analytical Forced Degradation/ Stress Studies

In Discovery

Stress studies including pH stability, physical stability, hot spot assessment, etc. to select the **best molecule** and identify development **risks**

In Clinical Development

Characterization of the **physicochemical properties** of the molecule

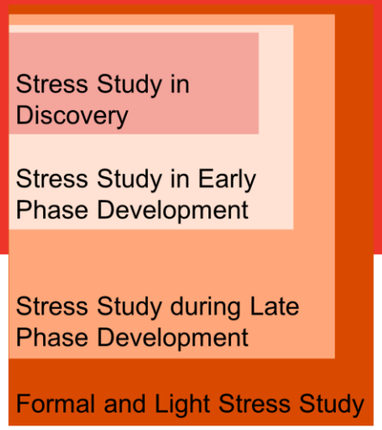
Elucidate degradation products, to support the determination of preliminary purity **CQAs and suitability of analytical methods**

Gather data to understand the **risk** to the manufacture, storage and commercial use of drug product and the need for formulation **strategies** for stabilization

For Registration

Stress and photostability studies to gather data required for the **impurities and stability** sections

In Discovery



Purpose:

- Select the **best molecule** and identify development **risks and potential molecule liabilities**

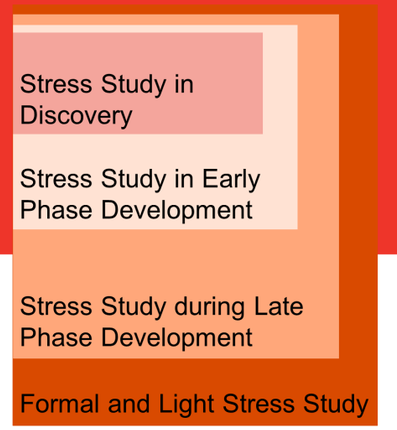
Stress Conditions:

- Molecule Degradation at high and low pH
- Temperature stress at a high concentration
- Light stress

Testing:

- Platform method conditions for stability indicating methods
- Peptide map analysis

In Early Phase Development



Purpose

- Augmentation of knowledge gained previously
- Assess analytical “platform” method capability and performance
- Determine pCQAs and define control strategy (e.g., develop a method to detect an oxidation in the CDR or support by peptide map) to support process and formulation development as well as stability testing of clinical material.

Design:

- Focusses on the key conditions that have been shown to be most informative from evaluation across multiple platform molecules
  - temperature, metal/peroxide, radical initiator
  - applied at different temperatures for 4 weeks plus light stress sample to streamline testing and reduce variability

Material:

- Any available matrix at a standardized concentration across the portfolio

Testing:

- Key impurity methods used for testing based on platform and molecule knowledge

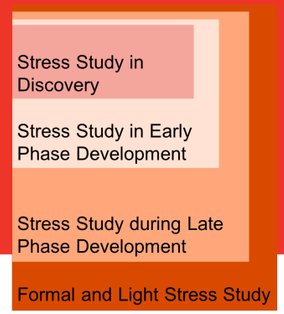
Early Phase (cont.)

- Example of key methods for a typical mAb:

Analytical Techniques	**Degradation Pathways Detected**
CE-SDS (Reduced and Non-Reduced)	Fragmentation, (aggregation), IgG1 hinge cleavage, IgG4 half antibody
Charge Heterogeneity (IEX-HPLC and/or icIEF and/or CZE)	Deamidation, citrylation, pyroGlu formation, succinimide formation, oxidation
SEC	Aggregation, (fragmentation)
Description (Visual)	Color (oxidation), particles (aggregation)
Bioassay	Inactive impurities, super potent species
SV-AUC	Aggregation, higher order structure perturbation

- Bioassay and mass spec analysis on selected samples
- SV-AUC to support SEC method development

In Late Phase Development



- Purpose:
  - Elucidate additional potential degradation pathways and assess the ability of the analytical methods to detect and quantify degradations in support of commercial process and formulation development
  - Identify stress condition appropriate to generate degraded samples needed for method validation
- Stress Strategy:
  - At least 2% to approximately 20% increase of total impurities/related substances
  - Significant degree of degradation that enables identification of the primary degradation products
  - Standardized testing design across molecules
- Material:
  - Development material regardless of small differences in cell culture or purification process (impact on rate rather than route)
    - Note: Changes in the cell culture or purification process leading to a change in the host cell protein profile could require a reevaluation of some conditions.
  - Drug Substance at 10 mg/mL in glass
  - Unbuffered, pH-adjusted water to show potential degradation pathways

Late Phase (cont.)

Stress Type	Typical Degradation Pathways Observed
Temperature	Oxidation, Isomerization/ Racemization, Aggregation, Fragmentation, PyroGlu
Oxidative	Oxidation, Aggregation, Fragmentation, IgG1 hinge cleavage
Metal-catalyzed	Oxidation, Fragmentation, IgG1 hinge cleavage
Metal+Peroxide	Oxidation
Radical Initiator	Oxidation
Acidic	Fragmentation, Isomerization/ Oxidation, Deamidation, Aggregation
Basic	Deamidation, Hydrolysis/ Fragmentation, Aggregation, Oxidation, Isomerization/ Racemization, Disulfide exchange, Beta elimination
Citrate	Citrylation
Glucose	Glycation
Dark Control	N/A
Light Exposure	Aggregation, Oxidation
Controlled Visible Light	Aggregation, Oxidation

Duration: 4 weeks at 5ºC, 25ºC and 40ºC plus initial sample

Late Phase (cont.)

Apply all methods that detect degradation products!

- Heavily Utilize Mass Spec:
  - LC-MS Peptide mapping to detect amino acid modifications at a low threshold
  - Non-reduced peptide map to investigate disulfide scrambling
  - If needed, use samples for peak isolation
- Submit for:
  - Bioassay methods: subpotent/superpotent species → CQA assessment
  - Biophysical methods: confirm SEC method performance

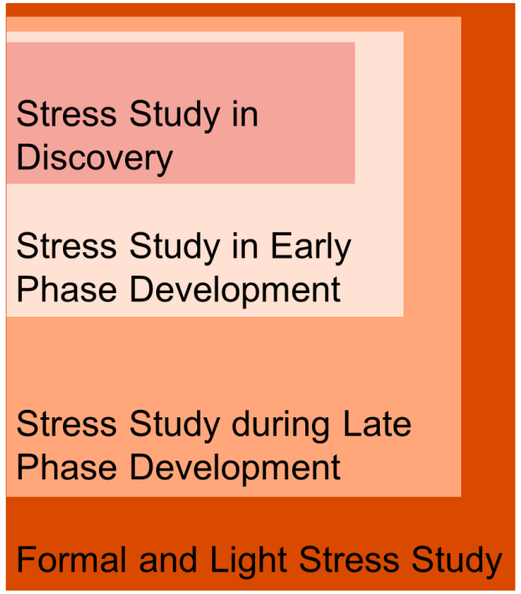
Applying the Knowledge Gained

Knowledge gained influences study designs and analytical support strategy

Examples:

- If heightened susceptibility towards oxidative stress is observed in the late phase stress study, the formulation screening would involve excipients that have been shown to be capable of providing protection against oxidative modifications (e.g., methionine, EDTA).
- If citrylation has been shown a potential degradation pathway, methods need to be included in the process and formulation studies that can identify those products, if citrate is used as a buffer
- If fragmentation is observed at low pH that cannot be detected by the platform CE-SDS method, additional methods need to be employed in the purification studies
- If aggregates are formed that cannot be detected by the standard SEC method, additional methods will have to be added to shear force or to shipping studies
- If there is no method to show a deamidation in the CDR that normally does not occur, additional methods will have to be employed when performing comparability studies

Formal Stress Stability Studies



- Follow the current ICH Q1A, Q1B, Q5C and other applicable regulatory guidelines
- Include degradation products that are typically observed during stability studies and/or impacted by the process conditions

- Define the list of key degradation products based on available data from previous forced degradation/ stress studies
- Select appropriate conditions that will demonstrate the capability of the analytical methods to detect these degradation products
  - Utilize the knowledge from earlier studies for choice of stress conditions
  - Ensure that the appropriate level of degradation is observed for each method, respectively (it might not be one stress fits all…)

Photostability Studies

- ICH Q1B provides minimum requirements, but not much guidance beyond….

- Three studies proposed:
  - Part I: Confirmatory Study (full exposure as per ICH Q1B)
  - Part II: Characterization of Light Exposure Study (investigating the impact of the manufacturing environment)
  - Part III: Stability Study Following Light Exposure

Thanks to

- Juliana Kretsinger
- Michael De Felippis
- Melody Gossage

Summary and Conclusion

- Stress studies are conducted to identify and/or confirm degradation pathways
- Samples are used to verify the appropriateness of the analytical methods used to support drug substance/ drug product development
- Throughout development, stress studies are important components in the determination/ refinement of CQAs and the definition of the analytical control strategy
- But remember: Forced Degradation is more than Stress Testing…