High Dose Delivery of Biologics
Development of Hyaluronidase Co-Formulations

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Presentation Outline

• Drivers for high dose formulations
• Introducing hyaluronidase as permeation enhancer
• Formulation development challenges
• Manufacturing process considerations
• Delivery device
Drivers for High Dose Formulations

- Requirement of high dosing for antibody drugs
- Intravenous (IV) infusion as typical administration route
- Subcutaneous (SC) injection as alternative administration route limited by volume (typically 1-2mL)
Drivers for High Dose Formulations

**Advantage of Subcutaneous Administration**

- Reduction of administration time and medical resource utilization benefits both HCPs, patients and the overall health care system
- Greater convenience for patients
- Self (home) administration:
  - SC complies with auto-injection devices
  - Beneficial for long-term/chronic therapies
  - Further cost reduction for health care systems
Drivers for High Dose Formulations

How to Overcome SC Volume Limitation?

- Approaches to delivering high antibody (mAb) doses subcutaneously
  - Increase mAb concentration
    - High-conc. liquids
    - Suspensions
  - Increase the dosing volume
    - Increase interstitial space by using hyaluronidase

Enhanze™ Technology is Halozyme’s platform for facilitating delivery of drugs using recombinant human hyaluronidase enzyme - rHuPH20
Introducing rHuPH20 as Permeation Enhancer

Hyaluronic acid (HA)
- large, repeating sugar polymer found in interstitial tissue
- forms a barrier to movement of molecules in the interstitial space

- Interstitial matrix limits SC injection
- Hyaluronidase is a naturally occurring enzyme that breaks down HA into tetrasaccharides
  - Permanent process (turn over > 5g/day in human)
- Co-administration with rHuPH20
  - Enables large volume SC administration of mAbs
  - Increases overall bioavailability (shortening Tmax, increasing Cmax)

Formulation Challenges

One Formulation Containing mAb and rHuPH20

• Development of a stable liquid high-concentration formulation of mAb

• mAb co-formulation with rHuPH20: keep two very different proteins in a stable and active state within one formulation (mAb in large excess, approx. 5000/1 on a weight basis)

• Avoid and demonstrate absence of detrimental interactions between mAb and rHuPH20

• Demonstrate homogeneity during compounding

• Test potential sensitivity of all formulation components, incl. rHuPH20, towards residual hydrogen peroxide (isolator filling technology)
Formulation Challenges

Approach for mAb – rHuPH20 Co-Formulation Development

- Scouting Study
  - pH/buffer screen
  - Surfactant screen
  - DoE (Design of Experiments)

- Stability Study with selected formulation candidates
Formulation Challenges

*Analytics for Co-Formulation Development Studies*

- Standard protein analytics
  - High and low molecular weight species (aggregates and fragments) by size-exclusion chromatography and SDS-PAGE
  - Charge heterogeneity by ion-exchange chromatography
  - Visible and subvisible particles
  - pH, osmolality, turbidity, color

- rHuPH20 analytics
  - rHuPH20 activity

- Characterization of high-conc. formulations
  - Viscosimetry
Formulation Challenges

Viscosity

- Viscosity influences
  - Syringeability
  - Functionality of auto injectors
  - Processing (e.g. homogenization, filtration, filling...)

Viscosity behavior of mAb

IgG1 at 150 mg/mL

- Most mAb formulations show pseudo-Newtonian fluid behavior
- Viscosity is a function of mAb concentration and excipients
- Viscosity needs attention during formulation development
**Formulation challenges**

**Viscosity Assessment of mAb & rHuPh20 Co-Formulations**

Rheogram of three mAb-rHuPH20 co-formulations at different temperatures

Viscosity (20°C, 2000s⁻¹) of mAb-rHuPH20 co-formulations (pH 5.5)

- The viscosity of all formulations was in an acceptable range with regards to processing and administration
Formulation Challenges

**Why Including Polysorbate 20?**

- Co-formulation of mAb with rHuPH20
  - mAb in large excess (weight ratio mAb/rHuPH20 approx. 1000 /1)

- Surfactant prevents mAb aggregation, concomitant with stabilization of rHuPH20 activity in the co-formulation

- Polysorbate 20 is more effective at lower concentrations than polysorbate 80
Formulation Challenges

*Why Including Methionine and Trehalose?*

- Methionine has a beneficial effect on mAb stability (e.g. levels of soluble aggregates) as well as on rHuPH20 stability (rHuPH20 activity, data not shown)
Formulation Challenges

*Is there Interference between mAb and rHuPH20?*

- Does rHuPH20 impact the glycosylation of the mAb?
  - Compare glycan distribution of the mAb in presence and absence of rHuPH20
  - Determine glycan distribution in presence of rHuPH20 over long term storage

- Side-by-side studies comparing mAb stability in the DP formulation with and without rHuPH20
  - Tested with routine and extended analytical methods
  - mAb stability also under stress conditions (temperature stress 30°C and initial light stress)
Formulation Challenges

*rHuPH20 Does not Impact mAb Glycan Structure*

- Relative glycan distribution was determined using CE with coupled fluorescence detection after enzymatic release of N-linked oligosaccharides

- No difference in the glycan structure abundance (%CPA) of drug substance (without rHuPH20) and of corresponding drug product (with rHuPH20)

- No significant change after storage of drug product at 2-8° C for up to 24 months
The mAb Stability is not Impacted by the Addition of rHuPH20

• The side-by-side comparison confirms that there is no significant change in the degradation profile of the mAb upon presence of rHuPH20
**Formulation challenges**

*No Direct Interaction between rHuPH20 and mAb was Observed*

- A Western Blot procedure in which rHuPH20 is detected was used to investigate potential formation of heteroaggregates.

- No additional bands detected during stability study for up to 24 months (2-8°C).
  - No aggregation products of rHuPH20 can be observed at the recommended storage condition.
Process Related Formulation Challenges

Compounding Operation

- Compounding process
  - rHuPH20 stock solution is added to the formulated mAb bulk
  - Challenge: the volume of rHuPH20 solution is comparatively low
    - Ratio 500/1 on a volume basis
    - Ratio 5000/1 on a protein weight basis

- Homogenization process was established using:
  - A formulated bulk surrogate solution having the same viscosity as the original mAb formulation
  - A marker simulating the rHuPH20 stock solution in a comparable small quantity
Process Related Formulation Challenges

Compounding Operation

- Samples were taken at different positions in the vessel and at different times after starting the homogenization procedure
- Samples were analyzed for marker content
- Criteria for assessment: relative content based on dilution (assuming homogeneity) and relative standard deviation

Marker (rHuPH20 surrogate)

T, M, B: sample locations

![Graph showing relative content over stirring time]
Process Related Formulation Challenges

Is there an Effect of Hydrogen Peroxide Residuals?

- Isolator technology is used for filling of vials
- Decontamination by means of vaporized hydrogen peroxide (VHP)
- Low levels of residual hydrogen peroxide may remain after aeration.
- It is known that residual amounts of hydrogen peroxide that are delivered into the filled drug product solution can impact the quality of the drug product
Process Related Formulation Challenges

*Is there an Effect of Hydrogen Peroxide Residuals?*

- The final drug product solution was spiked with different amounts of H$_2$O$_2$ stock solution -> subsequent stability testing
  - Appropriate oxidation assays (Pep-Map, RP-HPLC) were applied to test antibody and rHuPH20 sensitivity towards oxidation (in addition to standard stability indicating assays)
  - Peroxide content was determined

![Peroxide assay graph](image)

Peroxide content drops quickly in the formulation -> likely due to immediate reaction with formulation ingredients
Process Related Formulation Challenges

_Hydrogen Peroxide Residuals Did not Impact the Stability of rHuPH20 or mAb_

- No oxidation of antibody and rHuPH20 seen in presence of $\text{H}_2\text{O}_2$, even at concentrations largely exceeding the target level of VHP in the isolator

- No effect of $\text{H}_2\text{O}_2$ on other stability-indicating assays (data not shown)
Outlook: Single Use Injection Device

- Single use injection device for controlled sc administration of the co-formulation

- Additional investigations are required (such as compatibility of co-formulation with container, interaction with silicone...)
Summary

- SC administration motivated the development of a highly-concentrated mAb formulation containing rHuPH20

- A robust, liquid co-formulation showing acceptable stability behaviour with respect to both proteins was developed

- The presence of rHuPH20 in the formulation has no impact on the quality, stability and degradation pathway of the mAb

- Specific properties of the co-formulation necessitate attention at each drug product development stage
  - Special care was taken to address potential issues regarding the compounding step (very high mAb/rHuPH20 ratio) and regarding sensitivity of the co-formulation towards decontaminating agents
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