1. SUMMARY

This protocol describes the experiments planned for the process characterization for the anion exchange chromatography step used in the hRS7 mAb intermediate downstream process. Prioritized process parameters were identified based on the severity score from a Failure Modes and Effects Analysis (FMEA). The three parameters related to resin lifetime and process intermediate stability will be evaluated in separate protocols. An augmentation of the fractional factorial design of experiments (DOE) into a response surface model design will be executed to evaluate equilibration/wash solution pH, equilibration/wash solution sodium chloride molarity, load pH, load sodium chloride molarity, and load ratio with respect to both product quality and process performance attributes. Data from the DOE will be analyzed based on multiple linear regression models using JMP 16 statistical software (SAS Institute). The results and associated models will be used in further analysis for classification of process parameters and definition of acceptable ranges.

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2. INTRODUCTION

hRS7 is an anti-human Trop-2 humanized IgG1κ monoclonal antibody expressed from a genetically engineered Sp2/0 (murine myeloma) cell line. In the Trodelvy (sacituzumab govitecan) antibody-drug conjugate, hRS7 is chemically conjugated via a peptide linker to SN-38, the active metabolite of irinotecan.

The anion exchange chromatography step is the second chromatographic step in the next generation hRS7 mAb intermediate downstream process. The anion exchange step uses Q Sepharose Fast Flow resin from Cytiva operated in flowthrough mode. This step is used to remove impurities present in the ultrafiltration/diafiltration 1 (UF/DF 1) pool such as host cell proteins (HCP), host cell DNA, and residual protein A.

A risk assessment was conducted to identify and prioritize process parameters in the anion exchange chromatography step. Risk levels were assigned based on both general prior knowledge and process specific knowledge from development experiments and historic batch analysis available at the time of assessment. Prioritized process parameters were defined as any parameter with a severity score of 10. The prioritized process parameters are considered potential critical process parameters (CPPs) and require further study to enable parameter classification and definition of parameter acceptable ranges. A design of experiments (DOE) study will be augmented to evaluate 5 prioritized parameters: equilibration/wash solution pH, equilibration/wash solution sodium chloride molarity, load pH, load sodium chloride molarity, and load ratio.

3. STUDY DESIGN

3.1. Objective

The goal for this process characterization study is to evaluate the effect of the prioritized process parameters on both product quality and process performance by augmenting a fractional factorial DOE into a response surface model design. Data from the augmentation will be analyzed based on multiple linear regression models using JMP 16 statistical software (SAS Institute). These results and associated models will be used in further analysis for classification of process parameters, and for definition of parameter ranges.

3.2. Study Rationale and Structure

The following process parameters will be tested with an augmented design with additional axial points and center points for conversion of a 2-level, resolution V, fractional-factorial DOE to a response surface model (RSM):

- Equilibration/wash solution pH
- Equilibration/wash solution sodium chloride molarity
- Load pH
- Load sodium chloride molarity
- Load ratio

Parameter ranges tested in this study will be equal to or wider than the intended manufacturing target ranges. The parameters, current manufacturing target ranges, and rationale for test ranges in this study are the same as in the previous DOE. The response surface model will be comprised of runs #1-20 in EXP23005145, "hRS7 Next Generation mAb Intermediate Anion Exchange Chromatography Process Characterization Study Protocol," and the augmented runs (runs #21-33) in this study summarized in Table 1.

Parameter evaluation will be performed using the response surface model design created using the JMP 16 software (SAS, Cary, NC). The design consists of a total of 20 fractional factorial runs and 13 augmented runs including ten axial points and three center point control runs added to the design to allow for evaluation of process consistency over the duration of the study. Run order was randomized. The detailed study design is shown in Table 2. Center point controls runs are listed in bold font.

Table 1. Rationale for testing ranges selected for DOE study

Parameter	UOM	Target	Target Range	Test Range	Rationale
Equilibration/wash solution pH	pH units	8.0	7.8 – 8.2	7.8 – 8.2	± 0.2 of target
Equilibration/wash solution sodium chloride (NaCl) molarity	mM	N/A	N/A	5 – 20	Test impact of expanded conductivity range from NaCl molarity
Load sodium chloride molarity	mM	N/A	N/A	5 – 20	NaCl molarity to bracket target conductivity range
Load pH	pH units	8.0	7.8 – 8.2	7.8 – 8.2	± 0.2 of target
Load ratio	g/L resin	Not defined	20 - 60	20 - 80	potential column loading density range

Table 2. DOE study design

Run #	Equilibration / Wash Solution pH	Equilibration / Wash Solution NaCl Molarity (mM)	Load pH	Load NaCl Molarity (mM)	Column Load Ratio (g/L-resin)
1	**8.0**	**10**	**8.0**	**10**	**50**
2	7.8	20	8.2	5	80
3	7.8	20	8.2	20	20
4	**8.0**	**10**	**8.0**	**10**	**50**
5	7.8	20	7.8	5	20
6	7.8	20	7.8	20	80
7	7.8	5	8.2	5	20
8	8.2	5	7.8	5	20
9	8.2	5	7.8	20	80
10	8.2	5	8.2	20	20
11	**8.0**	**10**	**8.0**	**10**	**50**
12	8.2	20	8.2	5	20
13	7.8	5	8.2	20	80
14	7.8	5	7.8	5	80
15	8.2	20	8.2	20	80

Run #	Equilibration / Wash Solution pH	Equilibration / Wash Solution NaCl Molarity (mM)	Load pH	Load NaCl Molarity (mM)	Column Load Ratio (g/L-resin)
16	**8.0**	**10**	**8.0**	**10**	**50**
17	7.8	5	7.8	20	20
18	8.2	20	7.8	20	20
19	8.2	20	7.8	5	80
20	8.2	5	8.2	5	80
21	**8.0**	**10**	**8.0**	**10**	**50**
22	**8.0**	**10**	**8.0**	**10**	**50**
23	8.0	10	8.0	10	20
24	8.2	10	8.0	10	50
25	8.0	10	7.8	10	50
26	8.0	20	8.0	10	50
27	8.0	5	8.0	10	50
28	**8.0**	**10**	**8.0**	**10**	**50**
29	7.8	10	8.0	10	50
30	8.0	**10**	8.0	10	80
31	8.0	10	8.0	5	50
32	8.0	10	8.0	20	50
33	8.0	10	8.2	10	50

3.3. Method and Materials

3.3.1. AKTA System Preparation

Studies will be performed on AKTA FPLC systems. Each AKTA system will be rinsed and sanitized using 1.0 M sodium hydroxide prior to use. Fit for use testing will be performed prior to study initiation to confirm that the system is suitable for use. The panel of system tests are as follows:

- Flow rate check to ensure pump flow rates are within specifications
- Sample pump test to check the functionality of the sample pump

- System test U9-M to check the functionality of the solvent delivery, system pumps, system pressure sensors, UV monitor and conductivity monitor

Upon successful suitability testing, the system may be used for up to four weeks without repeating the system testing.

3.3.2. Process Operations

The study will be performed using a scaled-down anion exchange chromatography operation. Chromatographic parameters, including phases and durations, linear velocity, buffer composition, and column bed height, will be kept equivalent to those used in the hRS7 manufacturing process described in REP-45902, "hRS7 Next Generation mAb Intermediate Downstream Process Description," except for the parameters detailed in Table 1. Column packing, integrity testing, and described anion exchange chromatography purifications will be performed using AKTA FPLC systems. All operations will be documented in a laboratory notebook.

3.3.2.1. Buffer Preparation

All buffers will be prepared according to the manufacturing-scale recipes, raw materials, and specifications, as described in REP-45902. Low and high pH equilibration/wash buffers will be titrated to achieve the target setpoints.

3.3.2.2. Chromatography Column Packing and Storage

The study will be performed using a 1.0 cm diameter Omnifit column body packed with naïve Q Sepharose Fast Flow resin to a target bed height of 20 ± 2 cm for a target column volume of 15.7 mL. Bed height and residence time will match the target ranges for manufacturing scale operations. The scale-down model column qualification specifications are detailed in Table 3. The column testing procedures and post-packing procedures will be performed according to Table 4.

Table 3. Column Qualification Specifications

Resin	HETP Specification	Asymmetry Specification
Q Sepharose Fast Flow	≤ 0.05	0.8 - 1.6

Table 4. Q Sepharose FF Integrity Testing and Post-Packing Procedures

Phase	Solution	Phase Duration (CV)	Flow Rate (cm/h)
HETP Equilibration	0.1 M Sodium Chloride	3.0	100
HETP Tracer	1.0 M Sodium Chloride	0.02	100
HETP Elution	0.1 M Sodium Chloride	2.0	100
Sanitization	1.0 M Sodium Hydroxide	2.0 Static contact time: 50 min	200
Equilibration	0.02 M Tris-HCl, 0.01M Sodium Chloride, pH 8.0	5.0	200
Storage	0.1 M sodium hydroxide	4.0	100

3.3.2.3. Feedstock

Frozen feedstock (UF/DF 1 Pool) sampled from Samsung Biologics, Commercial Campaign 6 and stored at ≤ -65°C will be used for this study. This feedstock was sampled from an hRS7 1.0 GMP run, which includes the Triton X-100 detergent viral inactivation and does not include the depth filtration operation. As a result, the impurity load is higher than expected for the Process D mAb process input. Prior to the study, the appropriate number of 12 L bags will be thawed. After thaw, the UF/DF 1 pool will be mixed and frozen in aliquots at ≤ -65°C until study initiation. Feedstock used in these studies is listed in Table 5.

Load material will be adjusted to target sodium chloride molarity by dilution with 0.02 M Tris-HCl, pH 8.0 or by spiking with 0.02 M Tris-HCl, 1.0 M Sodium Chloride, pH 8.0. Adjustments to target pH will be performed by titration with 0.5 N hydrochloric acid or 0.5 N sodium hydroxide.

Table 5. Feedstock information

Study	Feedstock Description, Feedstock Lot
DOE augmentation	UF/DF 1 Pool, Samsung Biologics, Commercial Campaign 6

3.3.2.4. Chromatography Process Parameters

The anion exchange chromatography operating parameters used in manufacturing are described in Table 6. The scale down operating parameters will match the manufacturing process as described in Table 6, except for the process parameters being varied according to the study design listed in Table 2. If multiple cycles will be performed, the run may proceed directly from

the resin wash phase to the equilibration phase. If the column will be held for >12 hours, the storage phase will be performed.

Table 6. Anion Exchange Chromatography Process Operations and Process Solutions

Phase	Solution	Phase Duration	Flow Rate (cm/h)	Flow Direction
Pre-Sanitization	1.0 M Sodium Hydroxide	2.0 Static Contact Time: 50 mins	200	Down
Wash	0.02 M Tris-HCl, 1.0 M Sodium Chloride, pH 8.0	2.0	200	Down
Equilibration	0.02 M Tris-HCl, 0.01 M Sodium Chloride, pH 8.0	5.0	200	Down
Load	UF/DF 1 pool	Range: 20 - 80 g/L resin Start Collection: 0.2 OD	200	Down
Elution	0.02 M Tris-HCl, 0.01 M Sodium Chloride, pH 8.0	To End Collection: 0.3 OD or 12 CV	150	Down
Strip	0.02 M Tris-HCl, 1.0 M Sodium Chloride, pH 8.0	3.0	250	Down
Post-Sanitization	1.0 M Sodium Hydroxide	2.0 Static Contact Time: 50 mins	200	Up
Regeneration	0.02 M Tris-HCl, 1.0 M Sodium Chloride, pH 8.0	2.0	250	Down
Resin Wash	WFI	2.0	250	Down
Storage	0.1 M Sodium Hydroxide	4.0	100	Down

3.3.3. Sampling and Analysis Plan

Aliquots of feedstock will be taken after filtration of the first thawed center point load samples and frozen in polypropylene cryogenic vials at ≤ -65°C. Additionally, aliquots of each load sample will be collected after titration and sodium chloride adjustment and filtration and frozen in polypropylene cryogenic vials at ≤ -65°C. Concentration, pH, and conductivity of each filtered, adjusted load will be measured. All testing will be completed for load material from one center point run. SEC, iCIEF, nrCE-SDS, and rCE-SDS testing will be performed once for each of the five load pH and conductivity combinations evaluated during the study to assess the impact of load adjustment on product quality. After the initial set of testing, additional testing of retains for adjusted and filtered load samples may be completed as needed.

Column eluates will be collected as single pools from each chromatography run, and concentration, pH, and conductivity will be measured. For each run, the pool aliquots will be frozen in polypropylene cryogenic vials at ≤ -65°C. Storage of any remaining pool material will be documented in an electronic lab notebook. Feedstock and sample aliquots will be submitted

for product quality analysis by analytical assays. The product quality attribute analysis plan and associated aliquot volumes for both feedstock and pools for the DOE study are listed in Table 7. All analytical testing will be conducted at Gilead Oceanside according to analytical test methods listed in Table 7.

Table 7. Sampling and analytical analysis plan for DOE Study thawed feedstock and generated pools

Feedstock / Pool	Aliquot Volume	Product Quality Attribute	Analytical Assay[1]
Load Material	1.2 mL	Residual Host Cell Protein	HCP ELISA, TM-546
	1.0 mL	Residual Host Cell DNA	qPCR, TM-544
	0.5 mL	Aggregate	SEC-HPLC, TM-541
	0.5 mL	Charge Heterogeneity	iCIEF, TM-538
	0.5 mL	Purity	nrCE-SDS, TM-539
	0.5 mL	Purity	rCE-SDS, TM-540
	0.5 mL	Concentration	SoloVPE (development measurement)
	10 x 0.5 mL	retain samples for future analytical testing, as necessary	
Adjusted Load Material	0.5 mL	Aggregate	SEC-HPLC, TM-541
	0.5 mL	Charge Heterogeneity	iCIEF, TM-538
	0.5 mL	Purity	nrCE-SDS, TM-539
	0.5 mL	Purity	rCE-SDS, TM-540
	0.5 mL	Concentration	SoloVPE (development measurement)
	10 x 0.5 mL	retain samples for future analytical testing, as necessary	
	0.5 mL	Aggregate	SEC-HPLC, TM-541

Anion Exchange Chromatography Pool	0.5 mL	Charge Heterogeneity	iCIEF, TM-538
	0.5 mL	Purity	nrCE-SDS, TM-539
	0.5 mL	Purity	rCE-SDS, TM-540
	1.2 mL	Residual Host Cell Protein	HCP ELISA, TM-546
	1.0 mL	Residual Host Cell DNA	qPCR, TM-544
	1.0 mL	Residual Protein A ligand	Protein A ELISA, TM-543
	0.5 mL	Concentration	SoloVPE (development measurement)
	20 x 0.5 mL	retain samples for future analytical testing, as necessary	

1. Test methods are in development. Sample preparation and analysis conditions may be adjusted to suit sample concentrations and matrices. Details for test execution will be captured in the laboratory notebook entries.

3.4. Data Analysis Plan

The performance of the chromatographic runs will be evaluated in terms of both product quality and process performance. Process performance evaluation will include qualitative evaluation of the chromatographic profiles. Wash volume, pH, conductivity, and concentration will be measured, and step yield will be determined. Product quality will be evaluated based on the test plan defined in Table 7.

The data from the study will be analyzed based on multiple linear regression models using JMP 16 statistical software. A model for each response will be created by including all main effects, two-factor interactions and quadratic interactions and then performing backwards stepwise reduction using a p-value cutoff of 0.05 while following effect heredity. The reduced model will be fit using standard least squares.

The results from this study and the associated models will be used in further analysis for classification of process parameters and definition of acceptable ranges.

3.5. Acceptance Criteria

Chromatography runs will be considered acceptable for analysis if they are carried out according to the procedures outlined in this protocol with any exceptions documented and if the control runs demonstrate consistent performance with respect to chromatographic profiles and step yield. All analytical assay data will be considered acceptable once reported.

4. EXCEPTIONS

All exceptions to this protocol will be documented at the time they occur. The study impact will be assessed and documented in the final report.

5. REFERENCES

Document No.	Document Title
EXP23005144	hRS7 Next Generation mAb Intermediate Anion Exchange Chromatography Process Risk Assessment (FMEA)
EXP23005145	hRS7 Next Generation mAb Intermediate Anion Exchange Chromatography Process Characterization Study Protocol
REP-45902	hRS7 Next Generation mAb Intermediate Downstream Process Description
TM-541	Determination of hRS7 (sacituzumab) 60 mg/mL Purity by Size Exclusion Chromatography (SEC)
TM-538	Determination of Purity and Identity of hRS7 (sacituzumab) 60 mg/mL by Capillary Isoelectric Focusing (cIEF)
TM-539	Determination of hRS7 (sacituzumab) 60 mg/mL Purity by Non-Reduced Capillary Electrophoresis – Sodium Dodecyl Sulfate
TM-540	Determination of hRS7 (sacituzumab) 60 mg/mL Purity by Reduced Capillary Electrophoresis – Sodium Dodecyl Sulfate
TM-546	Quantitative Determination of SP2/0 Host Cell Proteins (HCPs) in hRS7 (sacituzumab) 60 mg/mL by Cell Line-Specific (CLS) ELISA
TM-544	Quantitative determination of residual murine host cell DNA in hRS7 (sacituzumab) 60 mg/mL by qPCR
TM-543	Determination of Residual Protein A in hRS7 (sacituzumab) 60 mg/mL by ELISA

6. SIGNATURES

Author: Duc Nguyen, Senior Research Associate I, DSPD — Date: 26MAR2024

Approved by: Andrew Quezada, Senior Research Scientist I, DSPD — Date: 26MAR2024

TITLE: hRS7 Next Generation mAb Intermediate Anion Exchange Chromatography Process Characterization Study Protocol

REVISION HISTORY

Rev.	Effective Date	Author	Description of Changes	Justification for Changes
1.0	Refer to approval page.	D. Nguyen	New	New document