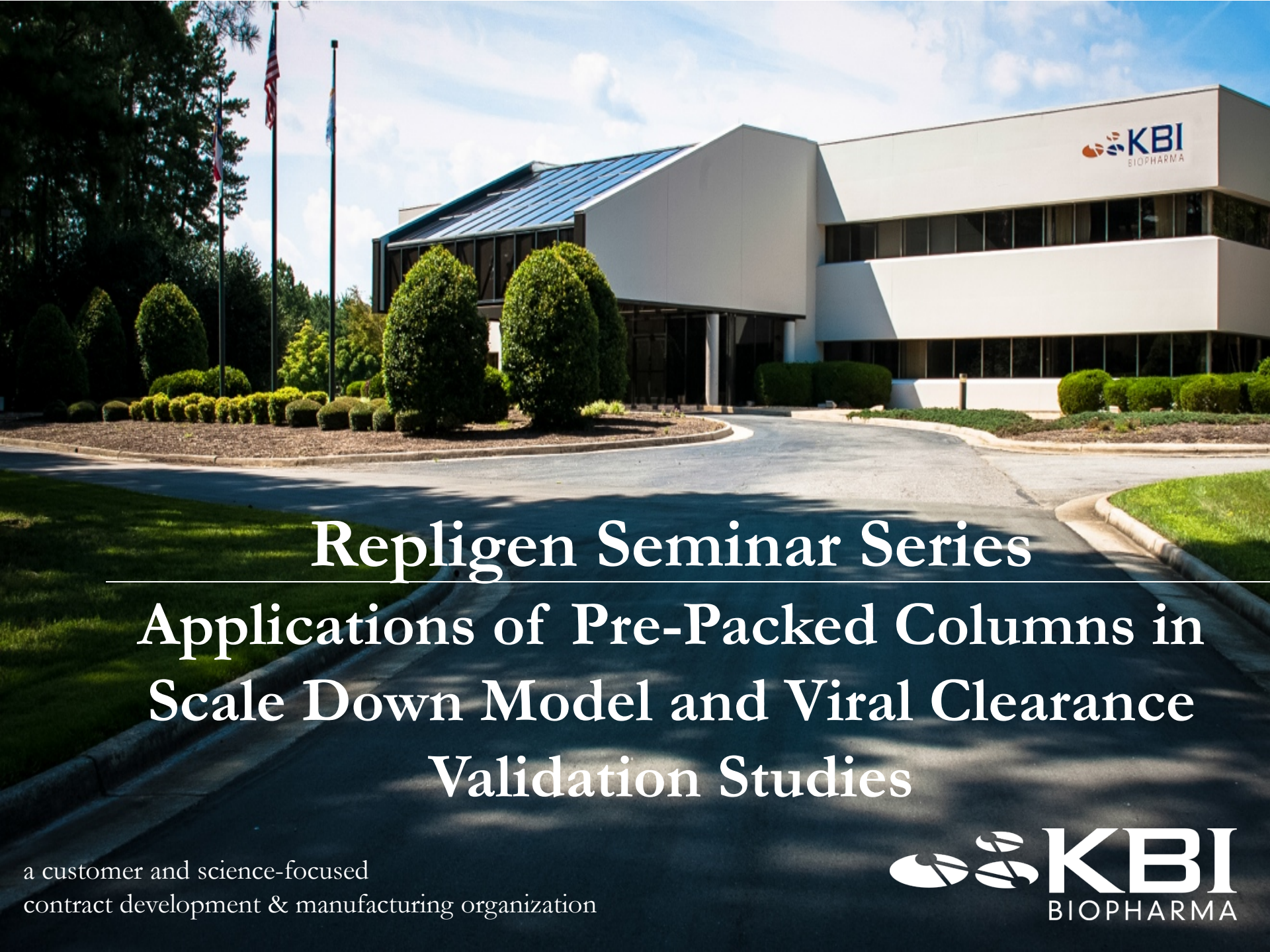


Repligen Seminar Series

Applications of Pre-Packed Columns in Scale Down Model and Viral Clearance Validation Studies

a customer and science-focused
contract development & manufacturing organization

KBI BIOPHARMA

KBI Biopharma: Who we are

-Contract Development and Manufacturing Organization (CDMO) founded in 1997

-Excellent track record of delivering cell line, upstream/downstream process, analytical and formulation development solutions for our clients on a variety of different molecules.

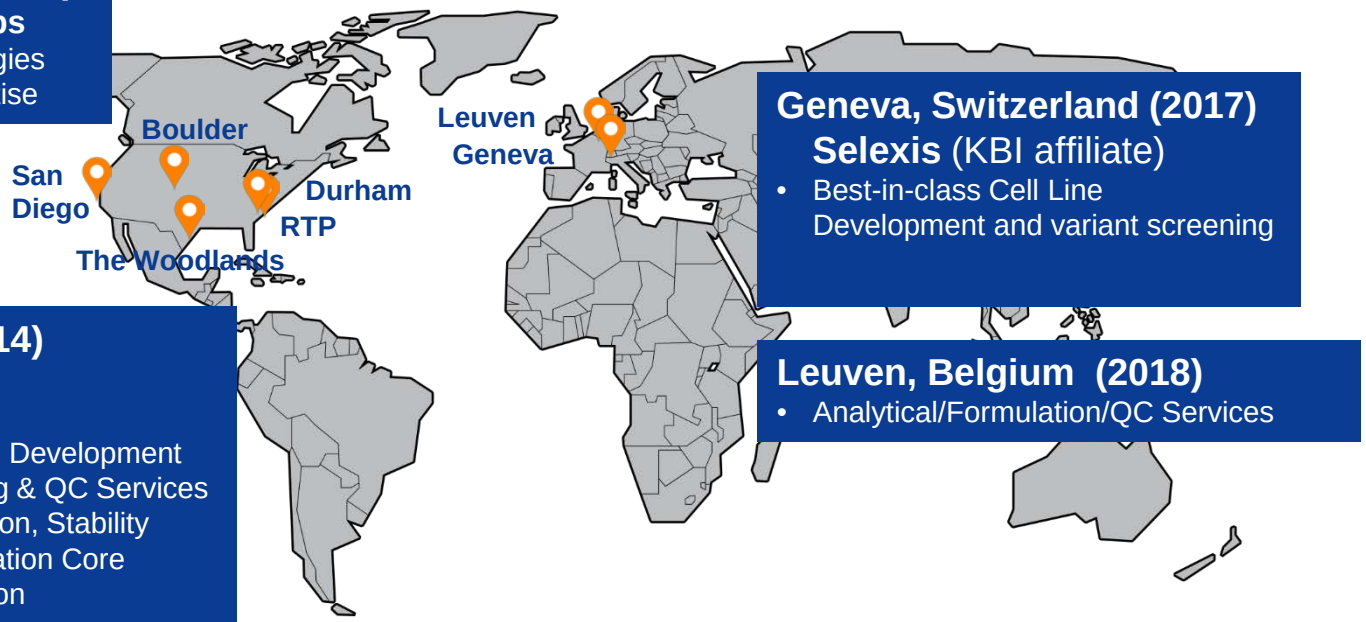
-Extensive experience with single-use biomanufacturing technologies

GMP manufacturing facility employs single use technology from seed train through bulk fill.

Where We Are- Global Presence

KBI Offices

San Diego, CA (2017)
Alliance Protein Labs
- Analytical Technologies
- Leading AUC expertise



Geneva, Switzerland (2017)
Selexis (KBI affiliate)
- Best-in-class Cell Line Development and variant screening

Leuven, Belgium (2018)
- Analytical/Formulation/QC Services

Boulder, CO (2014)
Microbial
- Strain Development
- Process & Analytical Development
- cGMP Manufacturing & QC Services
- Analytical, Formulation, Stability
- Particle Characterization Core
- Modeling & Simulation

Durham, NC (2004)
Mammalian
- Cell Line Development
- cGMP Manufacturing & QC Services
- Analytical, Formulation, Stability
- Mass Spec Core Facility

RTP, NC (2013)
Mammalian
- Process & Analytical Development
- Process Characterization
- Small scale Process Validation

The Woodlands, TX (2017)
Cell Therapy
- Process & Analytical Development
- cGMP Manufacturing & Testing
- Cell Based Assays

KBI BIOPHARMA

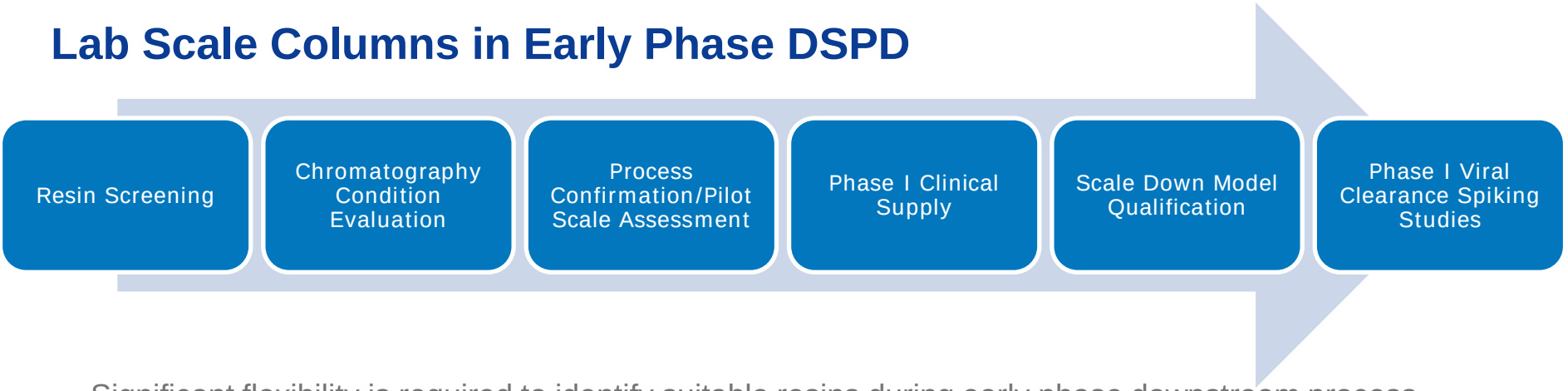
KBI-NC: Mammalian Process Development

- KBI has performed mammalian Process & Analytical Development for **50+ molecules**
- Successful on a wide variety of programs: enzymes, mAbs, vaccines and fusion proteins

  12+ client INDs supported per year
- Specific focus on First in Human mAb & non-mAb platforms **(10 bispecifics & >40 mAbs)**
- High-Throughput Process Characterization & scale down Process Validation
- PD using Selexis cell lines, client supplied cell lines, or full process ‘Transfer In’
- High-Throughput Analytics to support cell line selection & Process Development

This presentation describes our experience in moving from process development to viral clearance studies for proteins expressed in mammalian cell lines.

Lab Scale Columns in Early Phase DSPD



Significant flexibility is required to identify suitable resins during early phase downstream process development.

HTPD formats

Batch binding in resin screening plates

Opus RoboColumns (0.5 – 0.6 ml)

Fixed bed height columns

Opus Minichrom Columns (0.2 – 10 ml resin bed volume)

These options are suitable for identifying resins with desired selectivity,

Not suitable for accurate small scale modeling of the final purification process.

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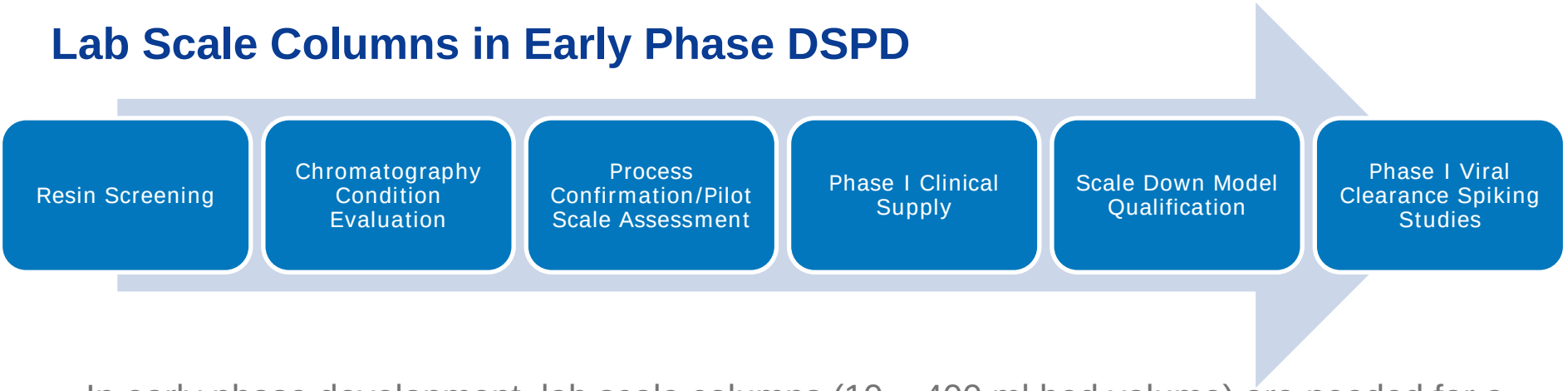
Lab Scale Columns in Early Phase DSPD



In early phase development, lab scale columns (10 – 400 ml bed volume) are needed for a number of studies once process resins have been selected.

- Chromatography Condition Evaluation
- Scale down model verification
- Viral clearance spiking studies

Later phase programs have additional studies that also require this scale of pre-packed columns

- Process characterization
- Resin lifetime studies
- Resin lot-to-lot variability assessments

Repligen Valichrom® Columns

Repligen Valichrom columns can provide a time-saving alternative to self-packed columns in house once DSP resins have been selected.

Advantages

0.5 – 2.5 cm internal diameters available provides flexibility for different bed volume needs

10 - 60 cm bed height range allows for flexibility to match large scale columns with different bed height specifications

Valichrom columns can be packed with resin from a number of manufacturers enabling standardization of scale down model column hardware

Each column is flow packed and tested individually

Statement of column performance characteristics provided with each column

Disadvantages

Because the columns are packed to customers specifications, lead time is typically 6 weeks which precludes their use in early process development/chromatography condition definition

Scale Down Model Assessment

At KBI, incorporation of Valichrom columns has been highly beneficial at the stage where we establish our scale down model to support viral clearance validation studies.

Scale down model assessment determines representativeness of lab scale operations relative to manufacturing scale operations

Model Input Factors	Model Output Factors
Column Diameter	Product Yield
Flowrate/Residence Time	Product Quality (Charge Species, HMW/LMW, Residuals)
Column Performance (HETP, Asymmetry)	Elution Volume
Buffer Variability	Elution Peak Shape
Process Intermediate Variability	

Scale Down model assessment involves performing multiple lab scale purification runs and comparing the results to manufacturing scale purification results.

Scale Down Model and Manufacturing Scale Data: Example 1

Parameter	Scale Down	GMP Production
# of runs represented by average values	3	3
Column ID	Valichrom 1.13 cm	Opus 45 cm
Yield (%)	72.3%	74.7%
%HMW (SEC-HPLC)	2.0%	2.3%
%LMW (SEC-HPLC)	0.3%	0.4%
%Heterodimer (CEX-HPLC)	100%	100%
Residual HCP (ppm)	33	32

Scale down model data compared to GMP data for a polishing step of a bispecific antibody

>1:1500 scale up factor between Valichrom columns and Opus GMP column

Scale down model effectively replicates chromatography step performance

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Scale Down Model and Manufacturing Scale Data: Example 2

Parameter	Scale Down	Pilot Scale	GMP Production
# of runs represented by average values	3	1	2
Column ID	Valichrom 1.13 cm	Opus 14 cm	Opus 45 cm
Yield (%)	98.2%	100%	94.7%
%HMW (SEC-HPLC)	4.5%	6.5%	3.6%
% Acidic Variants (WCX-HPLC)	43.5%	43.2%	41.2%
% Basic Variants (WCX-HPLC)	35.2%	31.8%	35.5%
Residual HCP (ppm)	1577	3177	1921

KBI Biopharma has also started to use Repligen pre-packed columns for pilot scale runs

Data shown is for product capture step

Bioreactor material was the same for the Scale Down and GMP runs

Pilot scale run material came from a different bioreactor

Considering expected process and assay variability, we have seen comparable scale up performance from lab to pilot and production scale Repligen columns.

Scale Down Model Takeaways

Scale down model verification using Valichrom columns has provided time savings relative to use of columns packed in-house.

Confirmation of scale down model involves comparison of process performance and product quality parameters from lab to production scale systems.

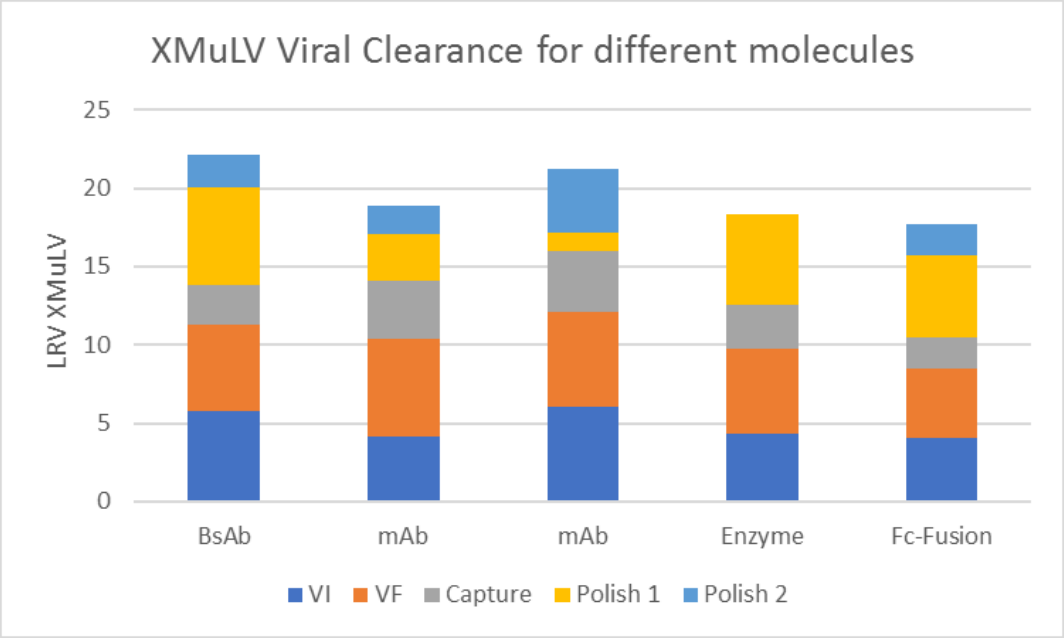
Scale down model can then be used to assess expected viral clearance in the DSP process through a viral clearance validation study

Viral Clearance Validation

Viral Clearance Validation targets a specific overall process Log Reduction of Virus (LRV)

Typically the total LRV required from a DSP process is in the range of 12 – 20 LRV.

Validation of viral clearance for at least one chromatography step will likely be required



LRV values in table are from specific DSP processes validated by KBI Biopharma Inc.
Not indicative of viral clearance for the indicated classes of molecules

Viral Clearance Validation

The scope of viral clearance validation studies depends on the status of the biologic in clinical trials

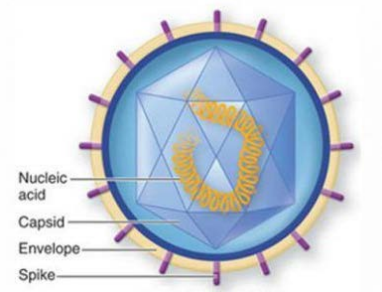
Phase I studies

- 2 different viruses (e.g. XMuLV, MMV)
- Duplicate Chromatography experiments

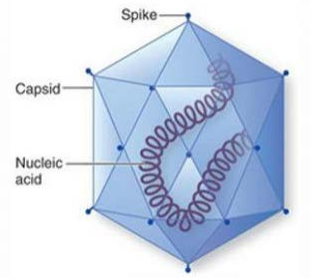
Phase III studies

- Expanded panel of viruses at least 4 (e.g. XMuLV, PRV, MMV, Reo3)
- Duplicate chromatography experiments
- Viral carryover assessment
- Viral clearance with aged chromatography resin

Regardless of clinical phase, the virus panel contains both larger enveloped viruses (e.g. XMuLV and PRV) as well as smaller non-enveloped viruses (e.g. MMV and Reo3).



Enveloped Virus



Non-Enveloped Virus

Viral Clearance Validation

Chromatography Steps Validated	Clinical Phase	Scale Down Columns	Virus Spike Run Columns	Total Columns Required
1	I	1	4	5
3	I	3	12	15
1	III	1	8	9
3	III	3	24	27

-Minimum column number accounts only for columns used for one scale down column and viral clearance spiking study (assuming new resin is used for each spike run).

-Additional columns may be required for repeating failed runs

-Phase III column requirements do not include needs associated with assessing viral

clearance with aged resin

Time Savings with Valichrom Pre-Packed Columns

In 2016, KBI Biopharma executed >12 viral clearance studies in support of our client's IND and BLA filings.

Phase I and Phase III studies were included in this assessment

Total columns required >200 columns.

At 3 hours per column to pack, assess, sanitize and store, this would amount to >**600 hours of time or >15 weeks of effort for 1 FTE for column packing**

Application of Valichrom columns for these studies represents significant time savings

DSPD scientists and associates can focus on more value-added process development activities

Repligen also offers packing services for client-supplied resins including aged chromatography resins from resin lifetime studies

Conclusions

Pre-packed chromatography columns represent a significant time savings once DSP process resins have been defined

Time savings is more significant as needs for lab scale columns increase in later stage programs (Process Characterization, Resin Lifetime Studies, etc…)

Valichrom column packing specifications and assessment results in columns with similar performance characteristics for scale down model and viral clearance validation studies.

Acknowledgments

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Questions?

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