MS-based host cell protein testing strategy for a vaccine manufactured in a novel cell line

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Melinda, *Tree of Life*
Melinda's artwork reflects her journey living with HIV.

Janssen Infectious Diseases & Vaccines
PHARMACEUTICAL COMPANIES OF Johnson & Johnson

Content

AdVac® vaccines produced in Per.C6® cell line

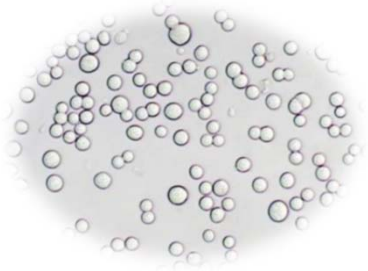
KFS Vaccines & host cell protein analysis

P V Mass spectrometry's contribution to HCP analysis

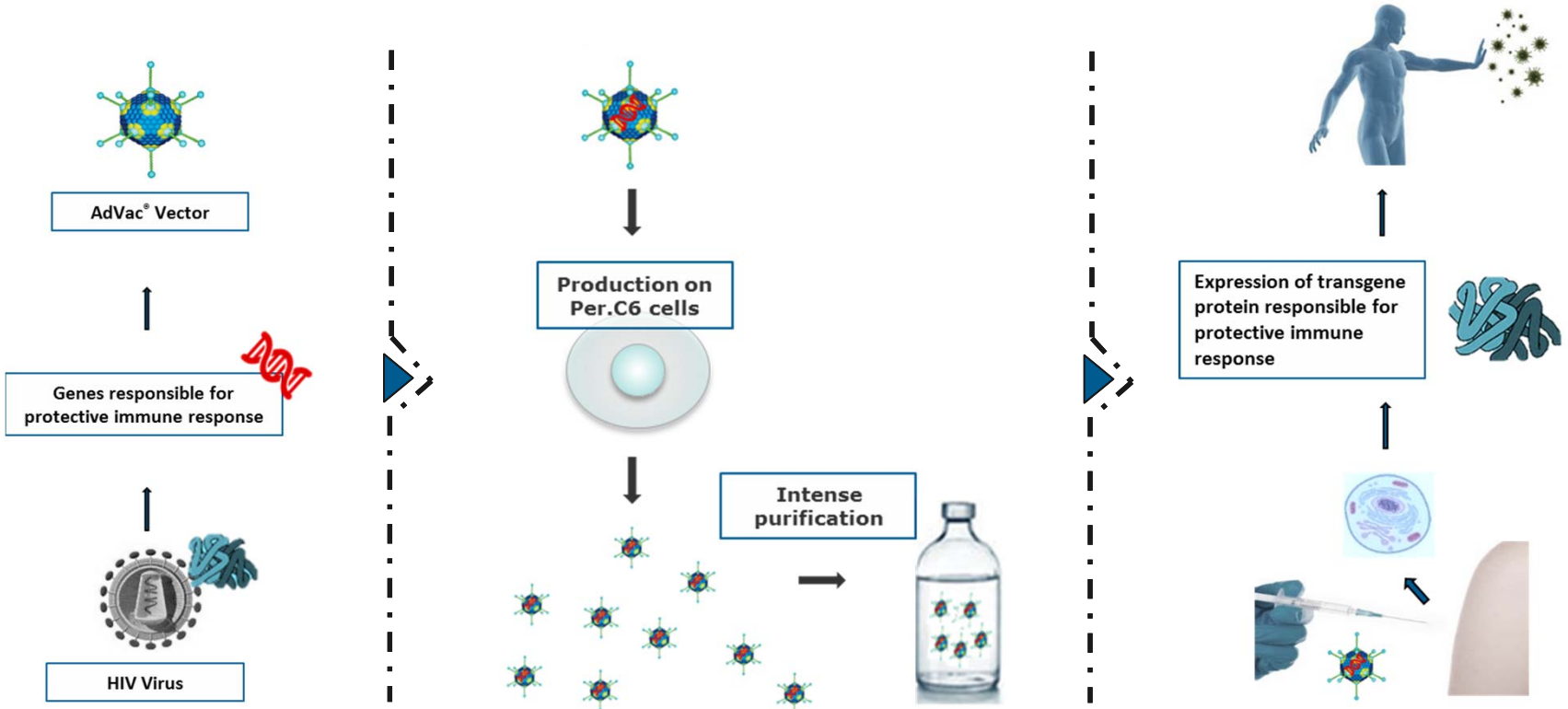
Vaccines form Per.C6® cell line

- Suspension cell line derived from human embryonic retinoblasts that grows at high density.

- Suitable for culturing of Janssen AdVac® platform at high virus titers.



AdVac® Platform: Principle



Vaccines & HCP: Guidance

- U.S. 21 CFR 610.13 requires a biological product to be "*…. **free of extraneous material** except that which is unavoidable…"*
- EP5.14 states *"The **concentration of residual HCP is determined** by a suitable immunochemical method…"*
- EMA/CHMP/VWP/141697/2009: "*a **test for residual HCPs** is needed"*.
- The FDA 2010 guidance for industry : "***The final bulk should be tested** for levels of **residual host cell proteins** to assure safety."*

Vaccines & HCP: Guidance

<USP 1132> raises three concerns regarding HCPs:

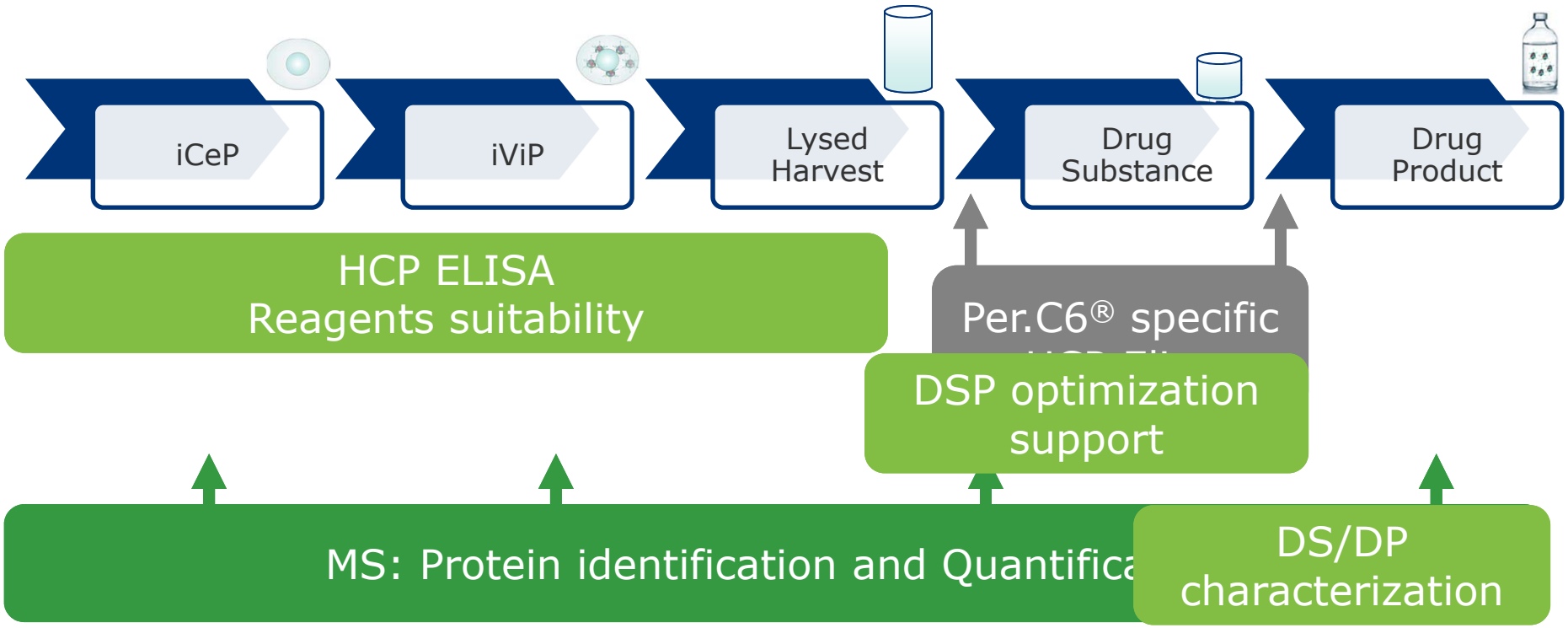
- **Immune reactions**: The potential to induce an immune response that could lead to an unwanted clinical effect in patients.
- **Adjuvant effect**: HCPs may act as adjuvants, which can enhance the formation of anti-drug antibodies that can affect the safety or efficacy of the API.
- **Bioactivity**: Bioactivity of HCPs may directly affect the critical quality attributes of the API.

HCP assessment AdVac® versus therapeutic protein

Overall the same, nevertheless different…

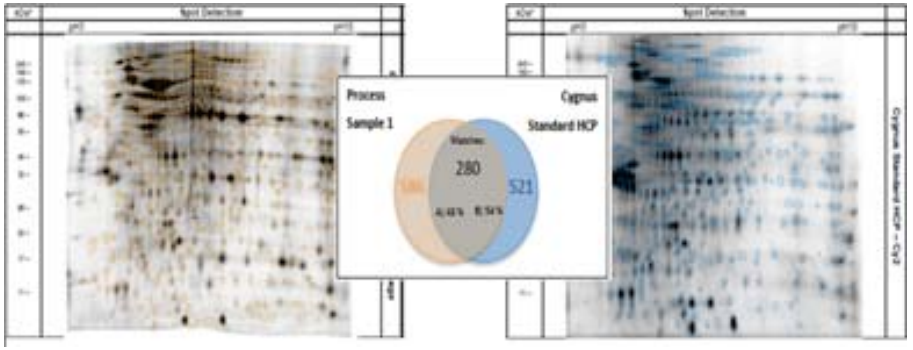
- Reversed mode of action as antibody, native HCPs, and limited administration reduce potential risk of HCP.
- Impossible to produce a null cell line, which increases the difficulty of proving the suitability of HCP ELISA.
- Protein concentration DS/DP AdVac® << DS/DP therapeutic protein.

HCP assessment AdVac® platform



Per.C6® specific HCP ELISA

- Release assay for all PER.C6® produced products.
- Developed by Cygnus using our proprietary cell line provided by Percivia in 2008 (iCeP).



- Validated as a generic assay for all current programs.
- HCP ELISA suitability assessment performed by comparative 2D electrophoretical analysis and affinity chromatography.
- What can mass spectrometry add to this?

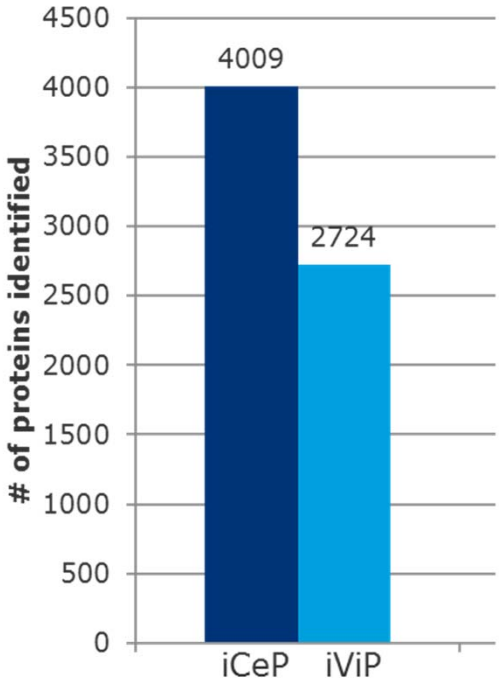
Proteomics approach

nLC/ESI-Q-Orbi:

- Tryptic peptides are obtained by FASP[1] on 30 kDa filter.
  - 8 M Urea denaturation,
  - DTT/IAA reduction/alkylation
  - LysC/Trypsin Digestion
- 3 h linear gradient on 75µm x 50 cm PepMap C18 EASY-Spray on Ultimate 3000 Nano + Q Exactive plus (Thermo Fisher Scientific) in DDA mode.
- Data interpretation by database search in human proteome (FDR 1% on both peptide and protein) with Protein Discoverer software (Thermo Fisher Scientific) based on spectral counting.
- ≥95% overlay in protein identity is accepted as identical protein composition.

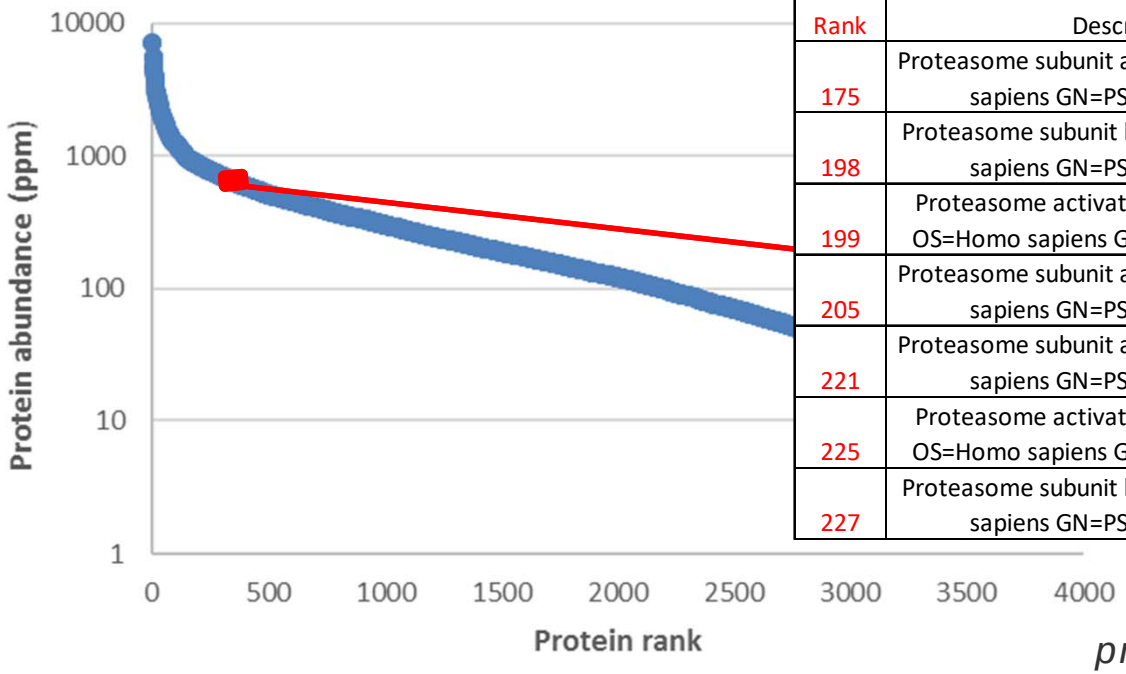
[1]Wisnieski et al, Nature Methods, 2009

iViP versus iCeP



- # protein identifications iViP « iCeP due to presence of high concentration of virus particles in iViP.
- Comparison of the 1000 most abundant proteins found in iViP to iCeP.
- **How to determine protein rank / abundance proteins?**

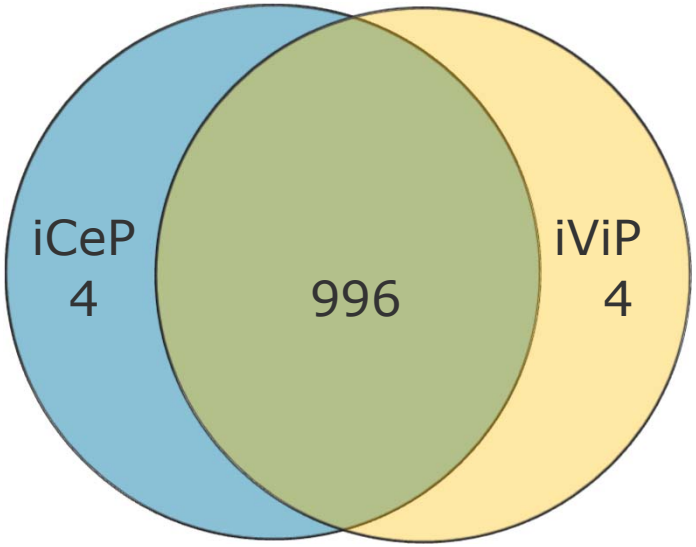
Protein quantification: Peptide spectral matches (PSM)

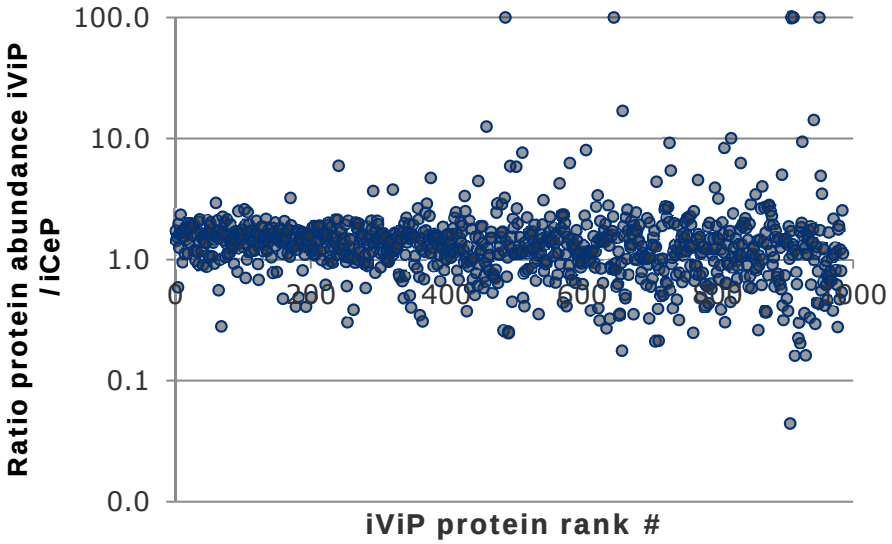


Rank	Description	# PSMs	# AAs	MW [kDa]	ppm
175	Proteasome subunit alpha type-7 OS=Homo sapiens GN=PSMA7 PE=1 SV=1	49	248	27.87	905.08
198	Proteasome subunit beta type-1 OS=Homo sapiens GN=PSMB1 PE=1 SV=2	44	241	26.472	855.64
199	Proteasome activator complex subunit 3 OS=Homo sapiens GN=PSME3 PE=1 SV=1	49	254	29.488	855.42
205	Proteasome subunit alpha type-6 OS=Homo sapiens GN=PSMA6 PE=1 SV=1	45	246	27.382	846.01
221	Proteasome subunit alpha type-5 OS=Homo sapiens GN=PSMA5 PE=1 SV=3	42	241	26.394	819.16
225	Proteasome activator complex subunit 2 OS=Homo sapiens GN=PSME2 PE=1 SV=4	43	239	27.384	808.35
227	Proteasome subunit beta type-7 OS=Homo sapiens GN=PSMB7 PE=1 SV=1	47	277	29.946	807.95

$$protein\ abundance^{1}\ (ppm) = \frac{\left(\frac{PSM}{MW}\right) x\ 1E^{6}}{Sum\frac{PSM}{MW}}$$

iCeP versus iViP: Protein composition

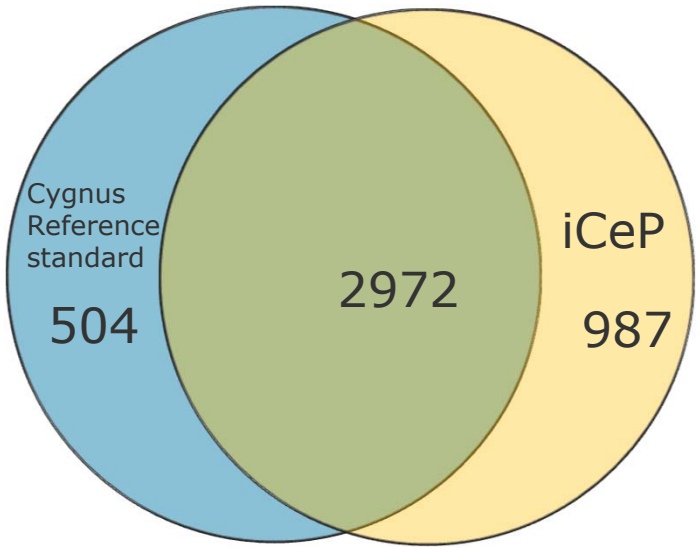




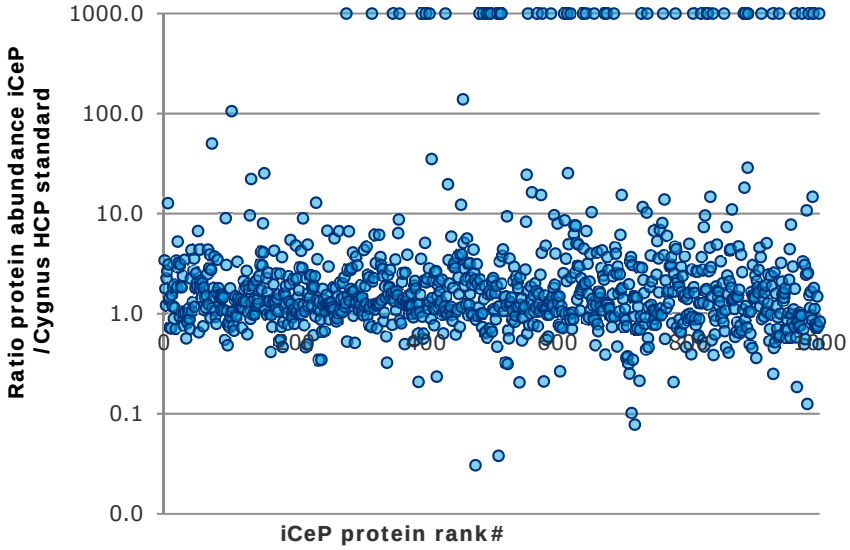
>99% overlap observed in top 1000 identified HCP.

Ratio abundance iViP/iCeP>100 protein not present in iViP

iCeP versus Cygnes HCP ELISA reference standard



95.0% match in protein identity

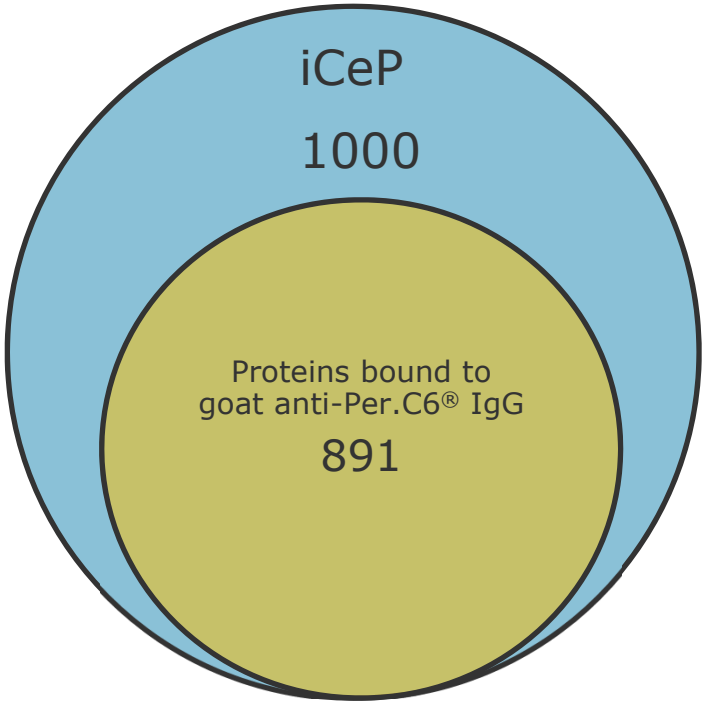


Ratio abundance iCeP/Cygnus HCP>100 protein not present in Cygnus standard

Cygnes Anti Per.C6® antibody coverage of iCeP

- Anti Per.C6 antibody's bound to affinity column.
- iCeP repeatedly run over column.
- Eluate (to affinity column bound proteins) analyzed by mass spectrometry.

- 89.1 % antibody coverage of top 1000 HCP Per.C6®.



Why determine HCP identity?

- To obtain optimal HCP clearance during down stream processing.
- Identity HCP present in DS enables the possibility to:
  - Assure coverage of HCP ELISA for these specific proteins.
  - Perform risk assessment based on <USP 1132> of HCPs identified in DS.

	HCP ELISA	LC-MS
Protein quantity	√	√
Protein identity		√
Assay status	Validated	Qualified for intended use
Assay throughput	high	medium

Mass spectrometric approach

µLC/ESI-Q-TOF:

- FASP, 30 kDa filter.
  - 8 M Urea denaturation,
  - DTT/IAA reduction/alkylation
  - LysC/Trypsin Digestion

- 1 h linear gradient C18 RP-µLC on 150µm x 10 cm HSS T3 Ikey M-Class + Synapt G2 (Waters) in MS^E mode.

- Data interpretation by database search in human proteome (FDR 4% combined with 2 peptide minimum) with Progenesis QI (Waters).

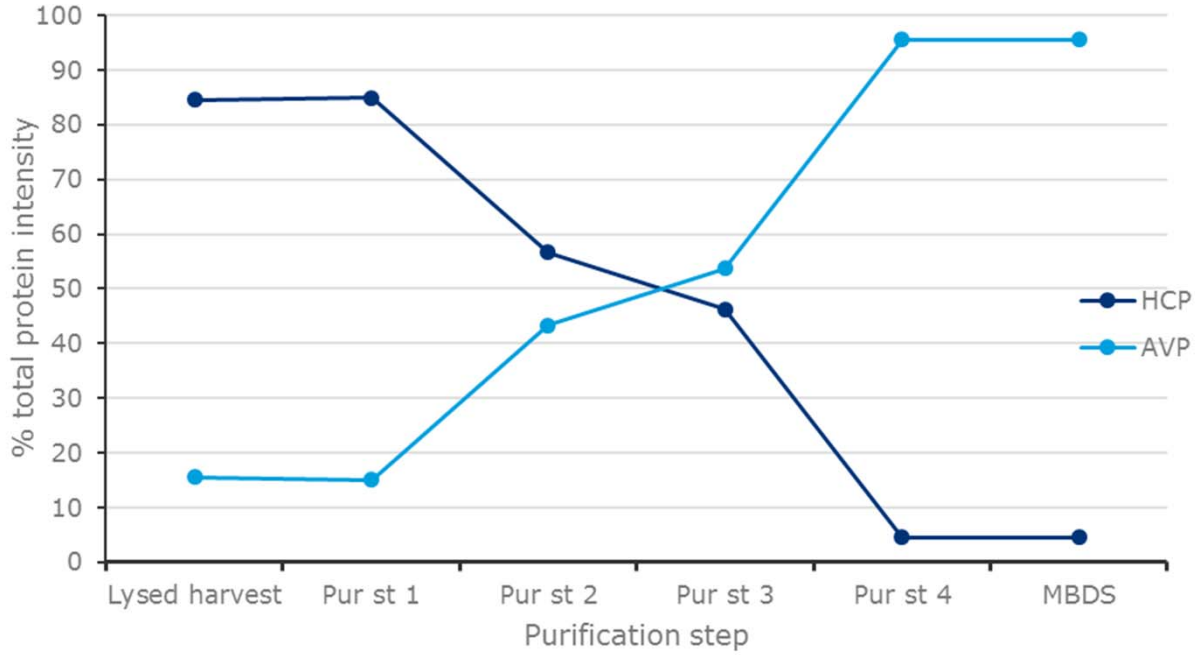
- Protein quantitation on H3 expressed as % of total protein intensity.

HCP clearance in down stream processing

Lysed Per.C6® cells

Multi step purification by chromatographic and size exclusion based separation

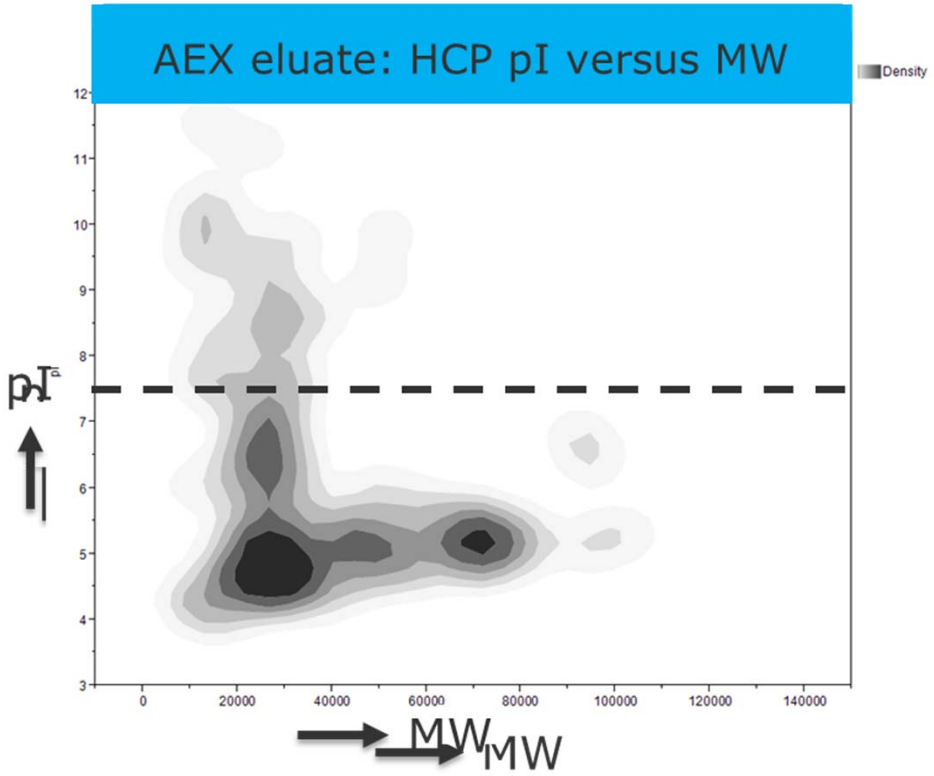
Monovalent bulk drug substance



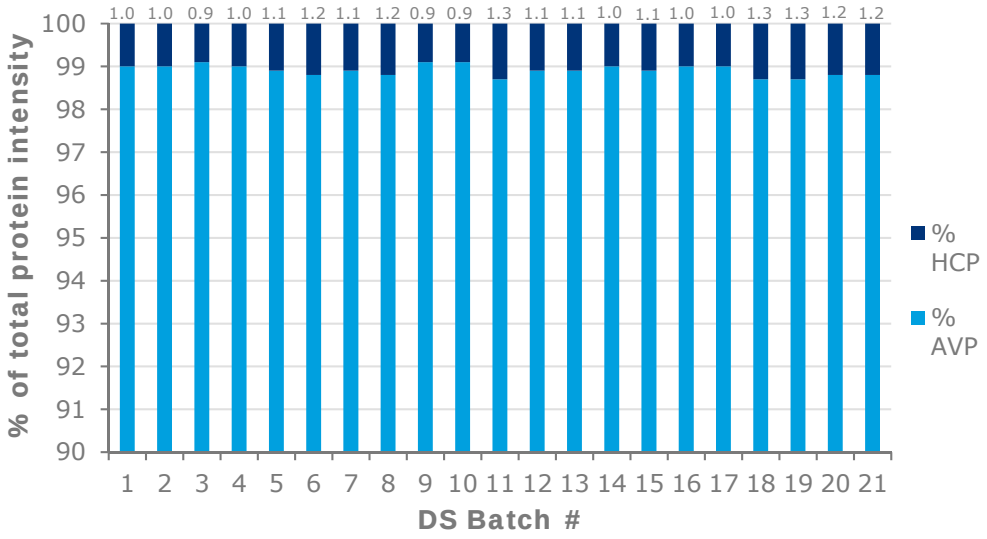
DSP optimization & HCP identity

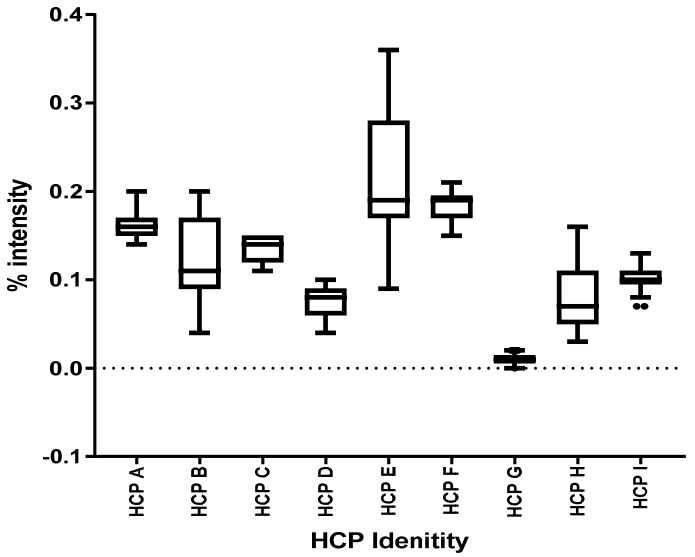
How can HCP identity be used for DSP optimization?

- HCP clearance by AEX Chromatography: Binding based on pI of protein.
- Only proteins with pI < 7.5 should bind on column.
- In AEX elute still proteins with pI>7.5, why?
- HCP identity gives knowledge about pI, MW, and structure.
- Most HCP with pI>7.5 are part of protein complex with pI<7.5 that binds on AEX column.



Drug substance charaterization:
Identification and quantification of residual HCPs (n=21)





- Consistent repertoire of HCPs in all batches
- Consistent HCP concentration per protein

Conclusion and future questions

LC-MS/MS is clearly an helpful tool in the analysis of HCP,

however…

- Can mass spectrometry replace the HCP ELISA?
- Mass spectrometry is a powerful and sensitive tool. What is the limit for identification of HCPs; when is information still relevant?

The People

Saskia Crowe
Jonathan Knibbe
Eveline Sneekes-Vriese
Shariteé Bouthisma
Arjen Scholten

And all colleagues of the analytical assay team of Janssen Vaccines & Prevention

Questions

Thank you

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14MAR19

Melinda, *Tree of Life*
Melinda's artwork reflects her journey living with HIV.

Janssen Infectious Diseases & Vaccines
PHARMACEUTICAL COMPANIES OF Johnson & Johnson