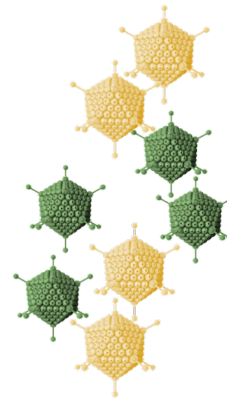


**EMA workshop on TTS
27.6.2022**

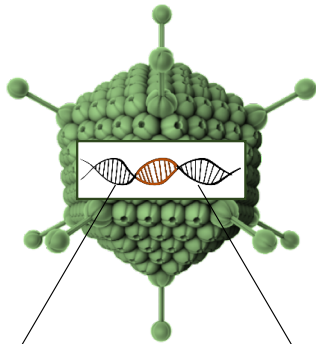
Process- and Product Related Impurities in the ChAdOx1 nCoV-19 Vaccine

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Adenovirus-based vaccines against SARS-CoV-2

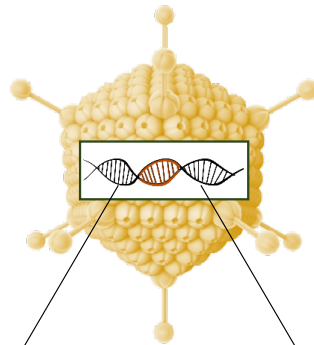
AstraZeneca
ChAdOx1 nCoV-2



SARS-CoV2-Spike

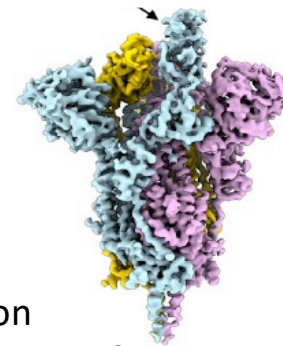
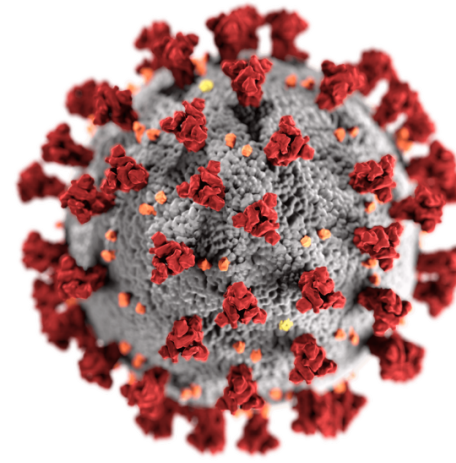
Chimpanzee
Adenovirus Y25
related to HAdV-E4

Johnson&Johnson
Ad26.COVS.S

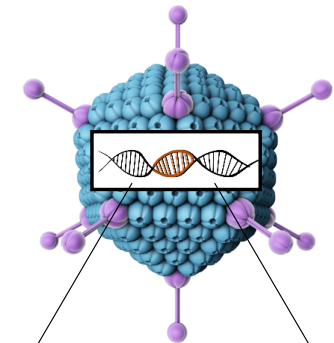


SARS-CoV2-Spike

Human
Adenovirus D26



UUIm
HAdV-C5-EGFP



EGFP

Human
Adenovirus C5

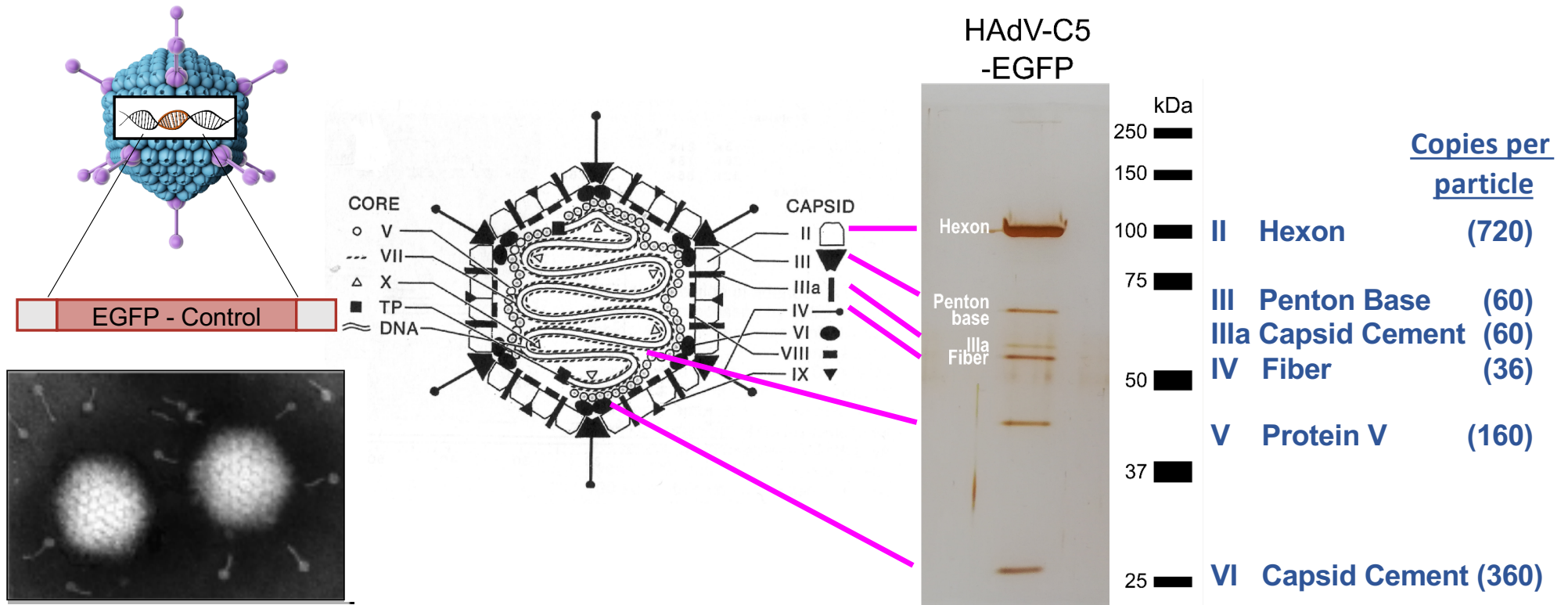


Control

- SARS-CoV-2 Spike = Antigen
- Induce good protective immunity against SARS-CoV-2 infection
- Well tolerated and broadly distributed vaccines with a very good safety record

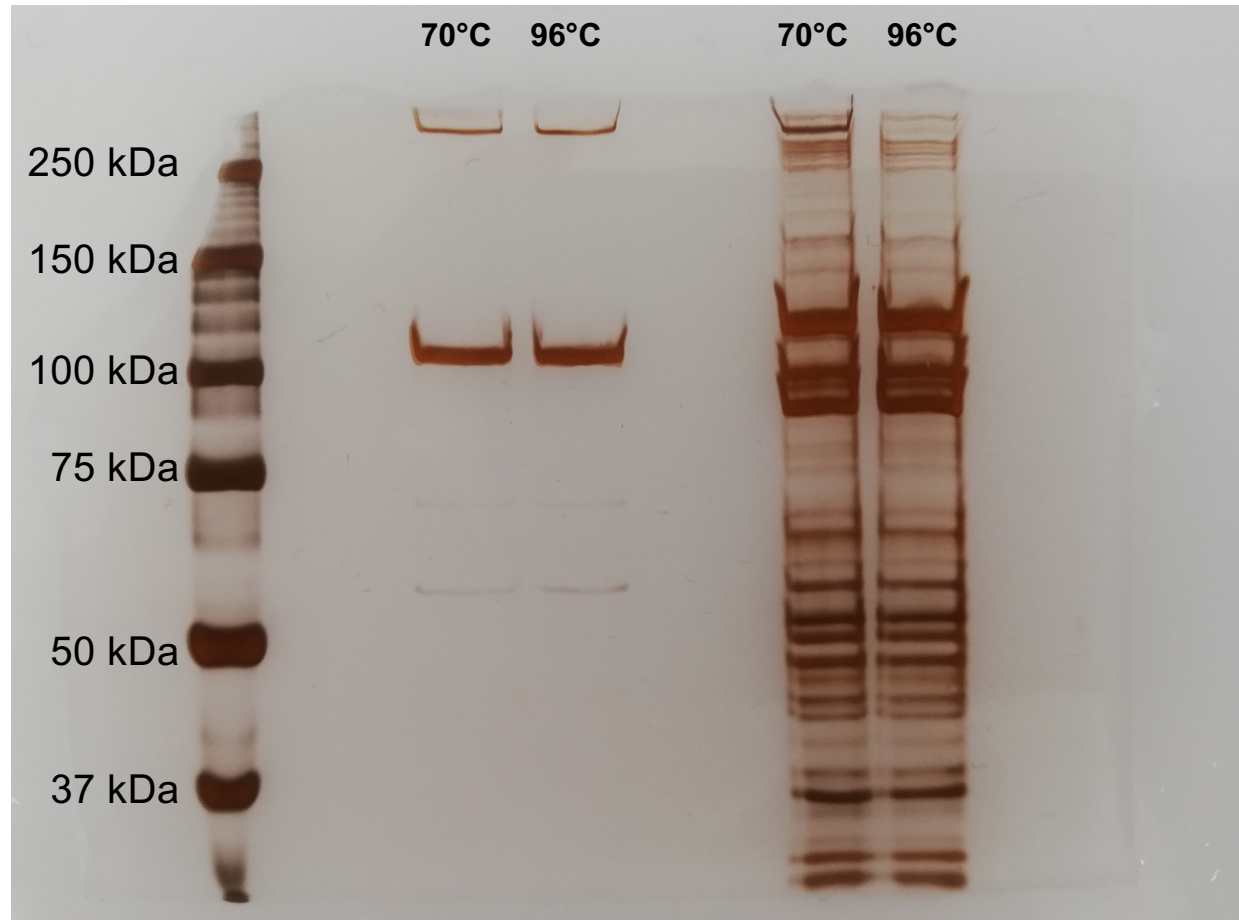
➡ **TTS: ChAdOx1 nCoV-2 in 1.6/100.000 -- Ad26.COVS.S in 0.755/100.000**

Standard assay after purification: PAGE / Silver Staining



29.3.2021 - HAdV-C5 versus ChAdOx1-S - PAGE/Silver staining

HAdV-C5 **ChAdOx1 Lot # ABV5811**
Gene Therapy, UUlM AstraZeneca



Titer determination

- HAdV-C5 - CsCl purified - OD260
- ChAdOx1 - used as per label

PAGE

- 3E9 vector particles in 30 μ l
- 5% stacking gel / 8% separation SDS gel
- Reducing conditions (SDS, β -mercaptoethanol)
- Samples heated for 5 min at indicated temperatures

Silver Staining

Repeat study: HAdV-C5 versus ChAdOx1 - three different lots

Titer determination

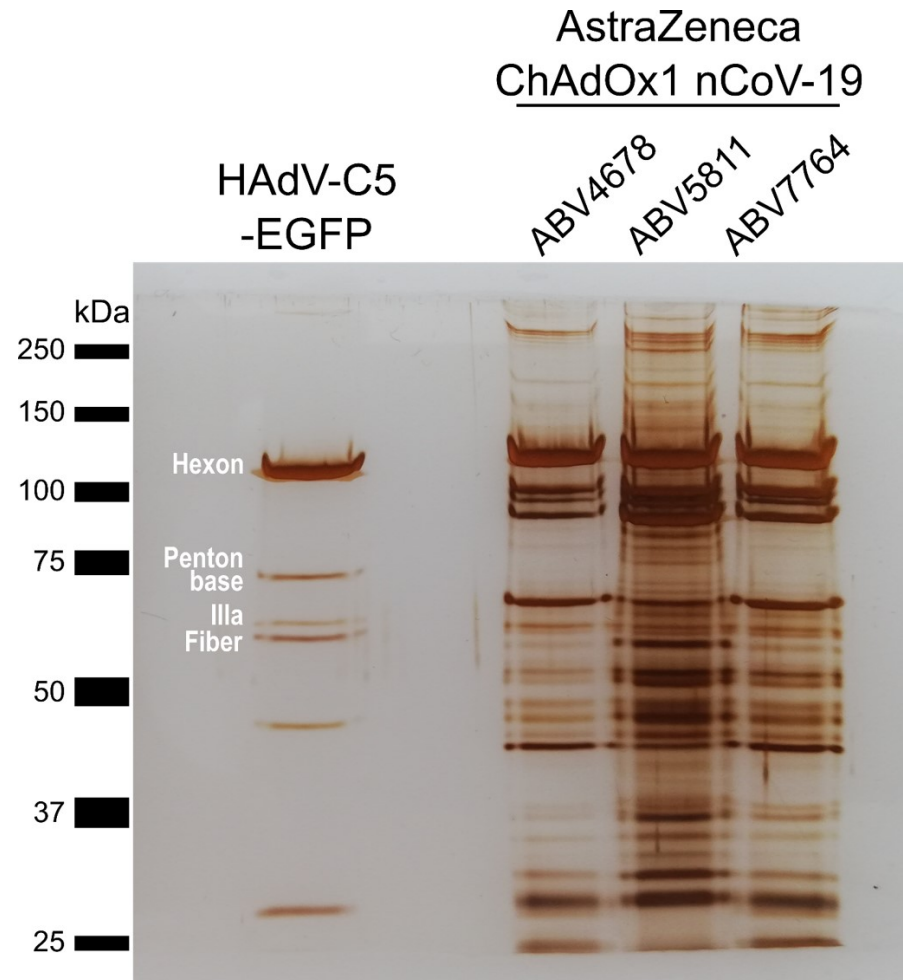
- HAdV-C5 - CsCl purified - OD260
- ChAdOx1 - used as per label

PAGE

- **3E9 vector particles** in 30 μ l
- 5% stacking gel / 8% separation SDS gel
- Reducing conditions
- (SDS, β -mercaptoethanol)
- Samples boiled for 5 min

Silver Staining

- ➔ - many unexpected bands
- substantial lot-to-lot variation

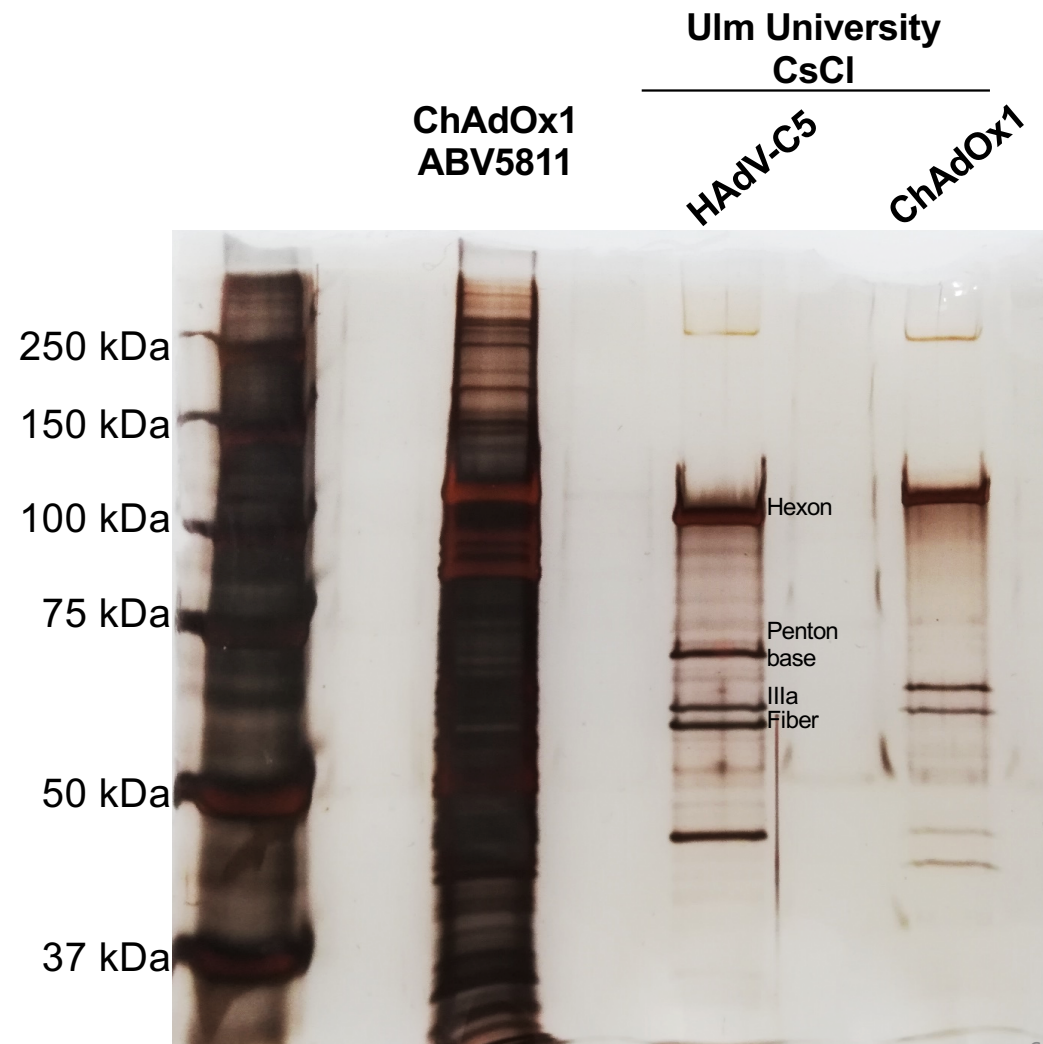


ChAdOx1 vaccine versus CsCl-purified HAdV-C5 and ChAdOx1

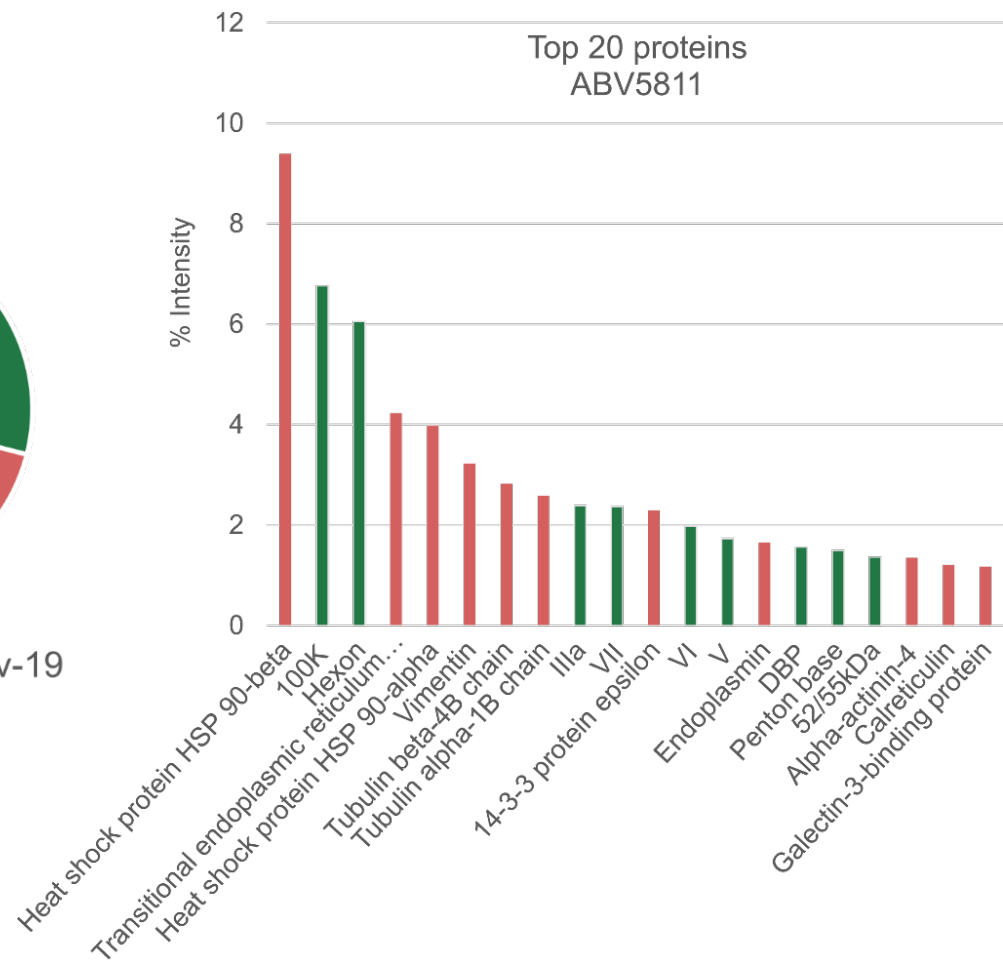
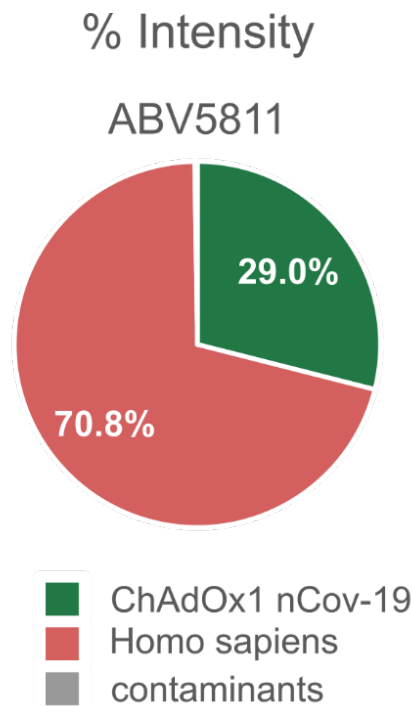
1. ChAdOx1 (vaccine) - AstraZeneca
2. HAdV-C5 (CsCl) - Ulm
3. ChAdOx1 (CsCl) - Ulm

Conditions

- 3E9 vector particles
- Reducing conditions (SDS, β -mercaptoethanol)
- 96°C / 5 minutes
- SDS/PAGE -> Silver staining



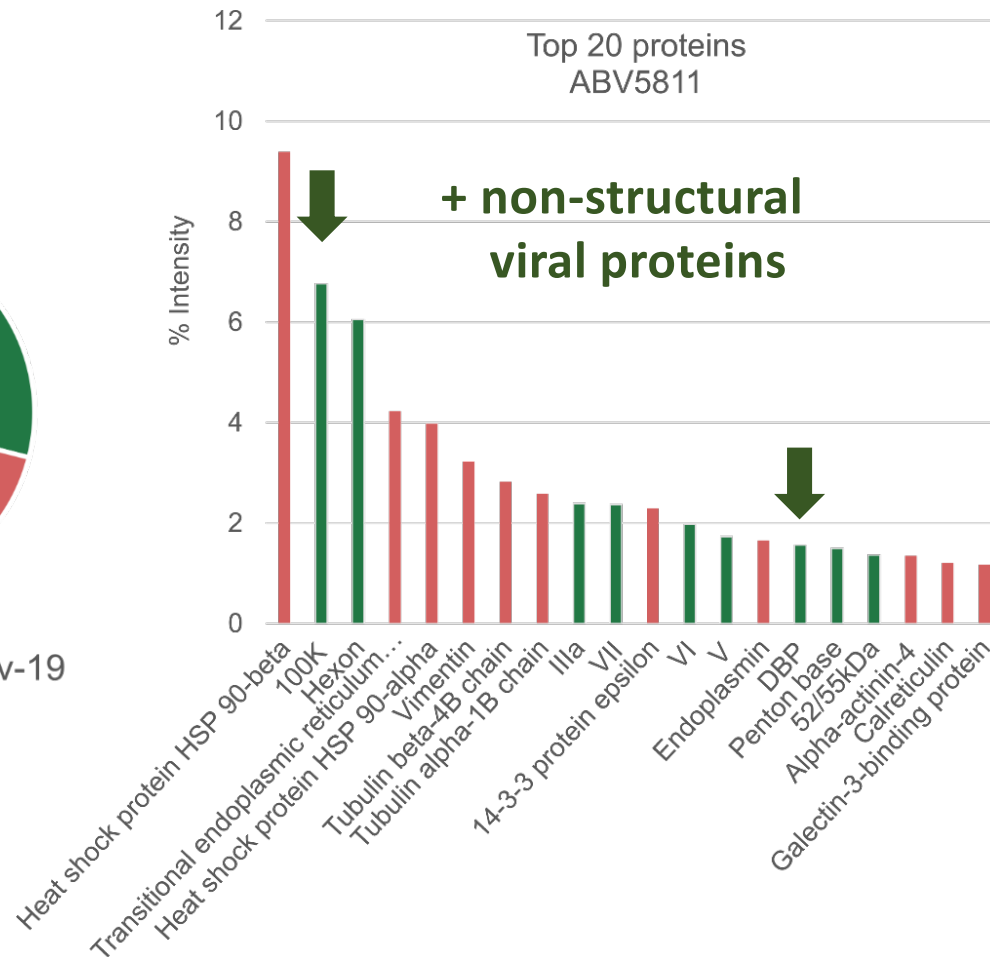
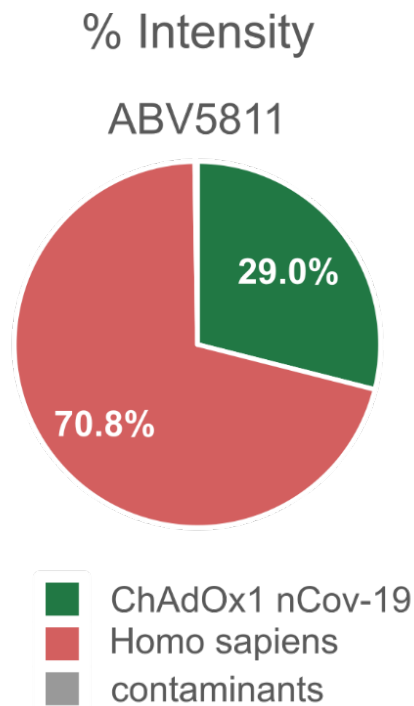
LC-MS analysis: more host cell proteins (HCPs) than adenoviral proteins



Dr. Reinhold
Rösler
Dr. Sebastian
Wiese

*Core Unit Mass Spectrometry
and Proteomics (CUMP)
Ulm University*

LC-MS analysis: more host cell proteins (HCPs) than adenoviral proteins

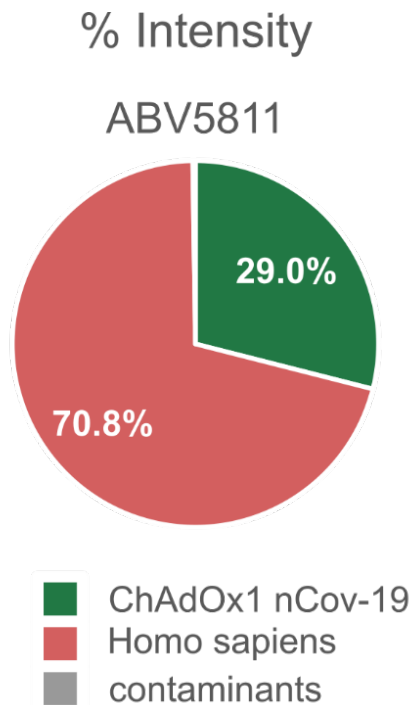


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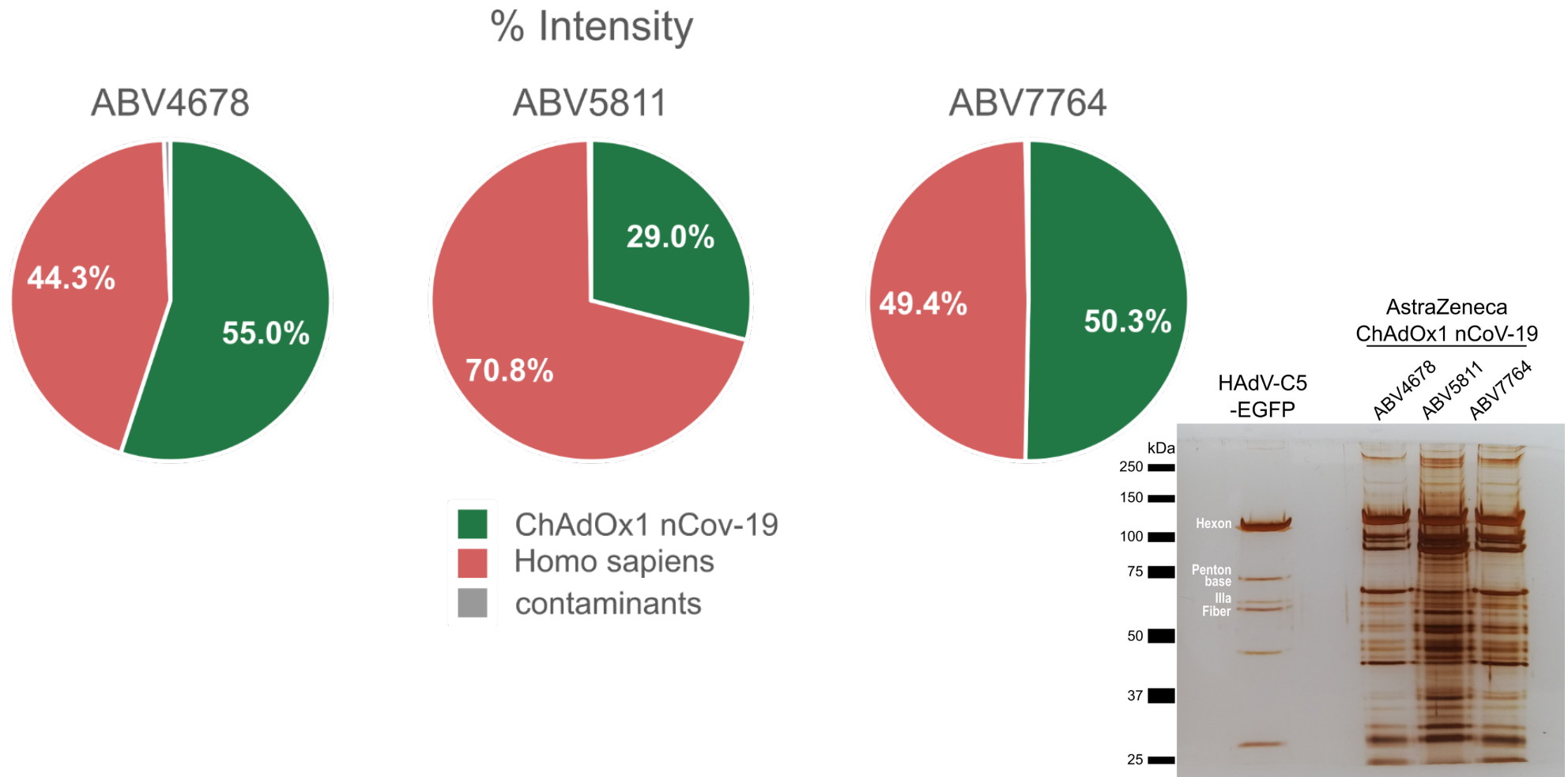
LC-MS - Top 30 proteins of the ChAdOx1 nCov-19 vaccine (ABV 5811)



Protein Name	Intensität %
Heat shock protein HSP 90-beta	9,48
100K	6,60
hexon	5,98
Transitional endoplasmic reticulum ATPase	4,26
Heat shock protein HSP 90-alpha	3,72
Vimentin	3,13
Tubulin beta-4B chain	2,82
14-3-3 protein epsilon	2,59
pVII	2,48
Tubulin alpha-1B chain	2,25
pIIIa	2,15
pV	1,77
pVI	1,69
DNA binding protein	1,68
Endoplasmin	1,60
penton	1,48
52/55kDa	1,42
Actin, cytoplasmic 2	1,24
Alpha-actinin-4	1,21
Calreticulin	1,19
Galectin-3-binding protein	1,04
Iva2	1,01
Glyceraldehyde-3-phosphate dehydrogenase	0,95
pIX	0,90
Nuclear autoantigenic sperm protein	0,74
60S acidic ribosomal protein P0	0,74
Elongation factor 1-alpha 1	0,72
pVIII	0,62
14-3-3 protein zeta/delta	0,62
Protein disulfide-isomerase	0,60

Heat shock proteins/chaperones
Cytoskeletal proteins
Adenovirus proteins

LC-MS: viral versus human proteins in three ChAdOx1 lots



Summary so far

- Significant protein impurities in ChAdOx1
- Lot-to-lot variation
- Higher human than viral protein content
- Presence of non-structural viral proteins
- Human proteins mainly HSPs and cytoskeletal proteins

ChAdOx1 - 5×10^{10} particles/dose

expected

12,8 µg

-> based on MW:

150 MDa

Protein amount (OD280) in ABV5811

measured:

32 µg

-> 20 µg mainly human proteins

Summary of functional data on ChAdOx1

Limited *in vivo* experiments in mice: original ChAdOx1 vaccine versus CsCl purified ChAdOx1

- No difference in anti-Spike antibody responses (ELISA)
- No difference in anti-Spike T-cell response (only 1 known epitope tested)

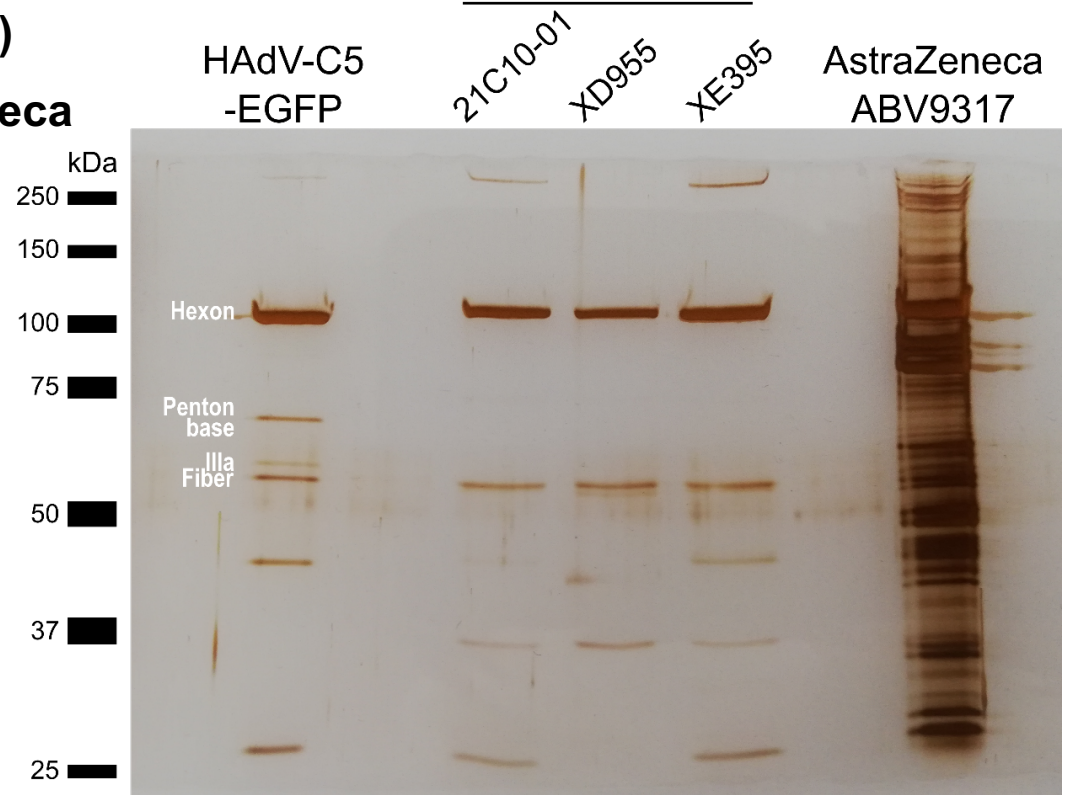
***In vitro* experiments**

- Enzymatic ATPase activity detected in ChAdOx1 -> likely derived from heat shock proteins

HAdV-C5 (CsCl) versus J&J Ad26.COV2 and ChAdOx1 vaccines

- | | |
|------------------------|---------------|
| 1. HAdV-C5 (CsCl) | - Ulm |
| 2. Ad26.COV2 (vaccine) | - JJ (3 lots) |
| 3. ChAdOx1 (vaccine) | - AstraZeneca |

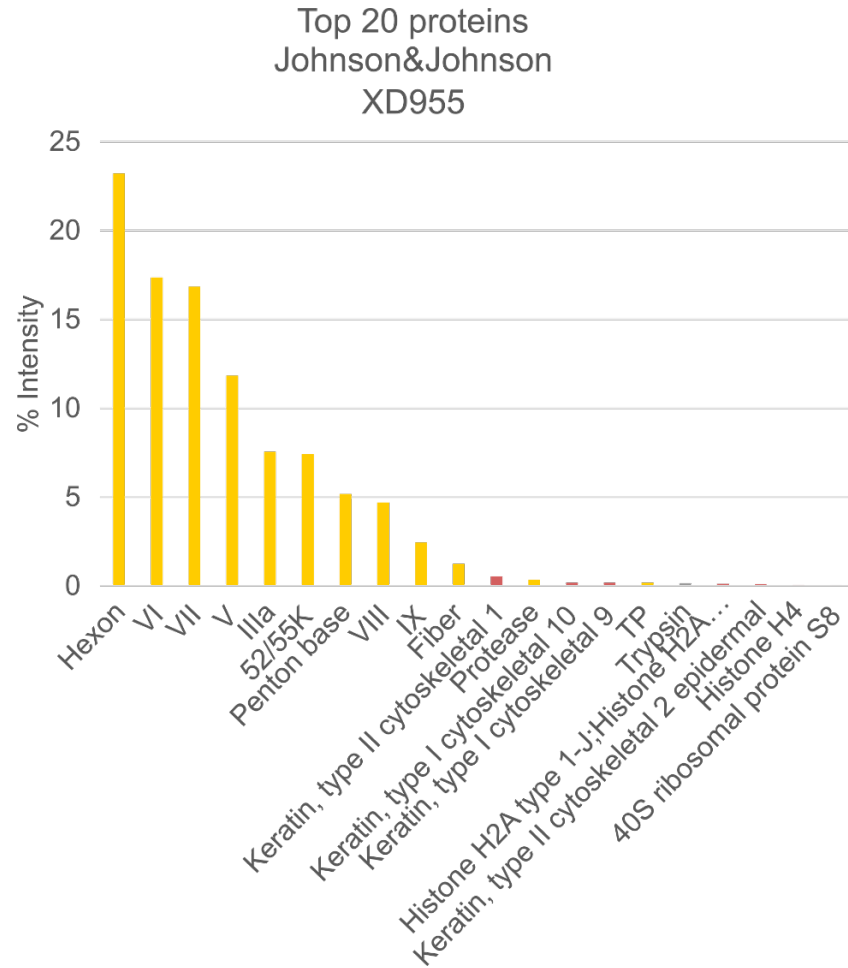
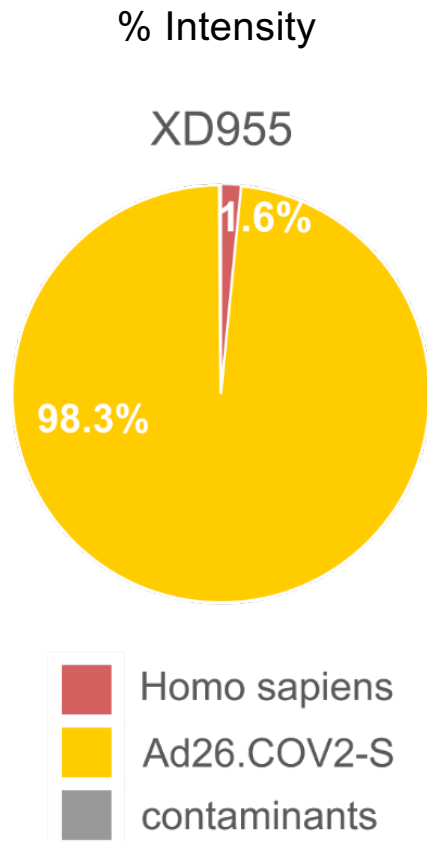
Johnson&Johnson
Ad26.COV2-S



Conditions

- 3E9 vector particles
- Reducing conditions (SDS, β -mercaptoethanol)
- 96°C / 5 minutes
- SDS/PAGE -> Silver staining

LC-MS: viral versus human proteins in Ad26.COV2-S lot XD955



➔ Very low levels of process- or product-related protein impurities in Ad26.COV2-S

EMA specification and batch release data: HCPs in 5 E10 ChAdOx1 particles (= 1 dose)

	EMA specification	Batch release data manufacturer	NanoDrop absorbance 280 nm	Bradford Assay	ImageJ analysis
	ChAdOx1 5 E10 particles [µg HCP/dose]	ChAdOx1 5 E10 particles [µg HCP/dose]	ChAdOx1 5 E10 particles [µg HCP/dose]	ChAdOx1 5 E10 particles [µg HCP/dose]	ChAdOx1 5 E10 particles [µg HCP/dose]
ABV4678	≤ 0.4	0.05	39.03	12.36	9.85
ABV5811	≤ 0.4	0.3	114.53	24.78	24.9
ABV7764	≤ 0.4	0.0489	74.37	29.66	12.81
ABV9317	≤ 0.4	n.a.	154.53	42.48	73.16

Protein amounts are after subtraction of 12.8 µg for 5 E10 viral particles

EMA specifications: Host cell proteins (HCPs) in 5 E10 ChAdOx1 particles (= 1 dose)

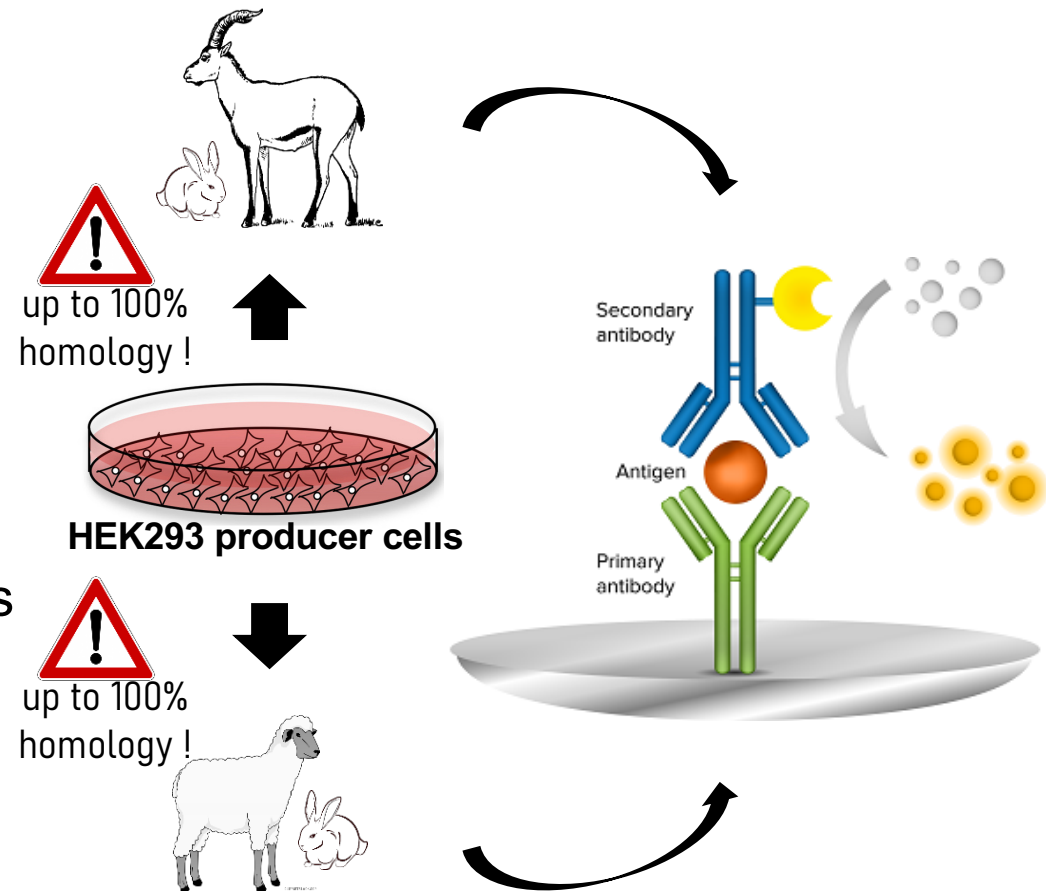
	EMA specification	Batch release data manufacturer	NanoDrop absorbance 280 nm	Bradford Assay	ImageJ analysis
	ChAdOx1 5 E10 particles [µg HCP/dose]	ChAdOx1 5 E10 particles [µg HCP/dose]	ChAdOx1 5 E10 particles [µg HCP/dose]	ChAdOx1 5 E10 particles [µg HCP/dose]	ChAdOx1 5 E10 particles [µg HCP/dose]
ABV4678	≤ 0.4	0.05	39.03	12.36	9.85
ABV5811	≤ 0.4	0.3	114.53	24.78	24.9
ABV7764	≤ 0.4	0.0489	74.37	29.66	12.81
ABV9317	≤ 0.4	n.a.	154.53	42.48	73.16



How to explain the discrepancy between batch release and actual data ?

Release assay for residual host cell protein (HCP) – Sandwich ELISA

➔ ELISA used likely
not suitable for detection of
HSPs and cytoskeletal proteins



Conclusions

Significant process and product-related impurities in 4 tested lots of ChAdOx1

- mainly HSPs/Chaperones, cytoskeletal proteins, non-structural viral proteins
- EMA specification for HCPs $\leq 0.4 \mu\text{g}$
- Lot release data $0.04 - 0.3 \mu\text{g}$
- Detected $> 10 - 50 \mu\text{g}$
- substantial lot-to-lot variation
- detection of enzymatic/ATPase activity
- in immunological pilot studies no difference between „dirty“ and „clean“ ChAdOx1

Very low HCP levels in 3 tested lots of Ad26-COV2-S (J&J)

Clinical implication

- Analysis of Ad26-COV2-S (also linked to SVT) suggests that the HCPs detected in ChAdOx1 are not the primary trigger of TTS/VITT (->no HCPs in Ad26-COV2-S – however TTS in 0.755/100.000)
- An adjuvant effect of the detected protein impurities in ChAdOx1 can currently not be excluded

Reason for discrepancy between release data and actual data

- ELISA used for batch release likely not suitable to detect HSPs & cytoskeletal (+ non-structural) proteins in ChAdOx1
- Assumption: ELISA used likely not validated with orthogonal assays

Needed

- Improved downstream processing/purification
- Suitable quality assays for complex biopharmaceuticals such as viral vectors

Acknowledgements

Dr. Lea Krutzke
Tatjana Engler
Ellen Allmendinger

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Ulm University*

Dr. Reinhild Rösler
Dr. Sebastian Wiese

*Core Unit Mass Spectrometry
and Proteomics (CUMP)
Ulm University*

For further details see the following accepted manuscript:

Krutzke, L., Rösler, R., Allmendinger, E., Engler, T., Wiese, S., Kochanek, S.
Process- and product-related impurities in the ChAdOx1 nCov-19 vaccine.
eLife 11 (2022) e78513. <https://elifesciences.org/articles/78513>

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