cytiva

Achieving vaccine equity

The challenges and opportunities of multi-modality manufacturing

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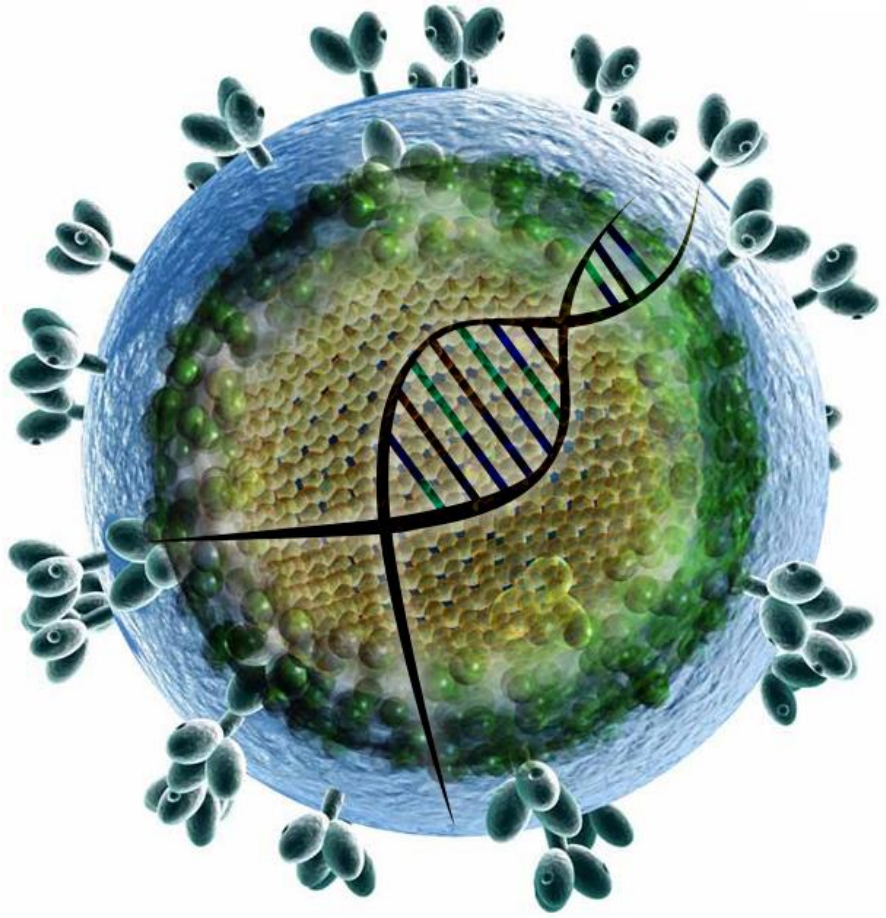
Agenda

- Introduction
- mRNA
- pDNA
- Viral vector
- Aseptic filling
- Manufacturability

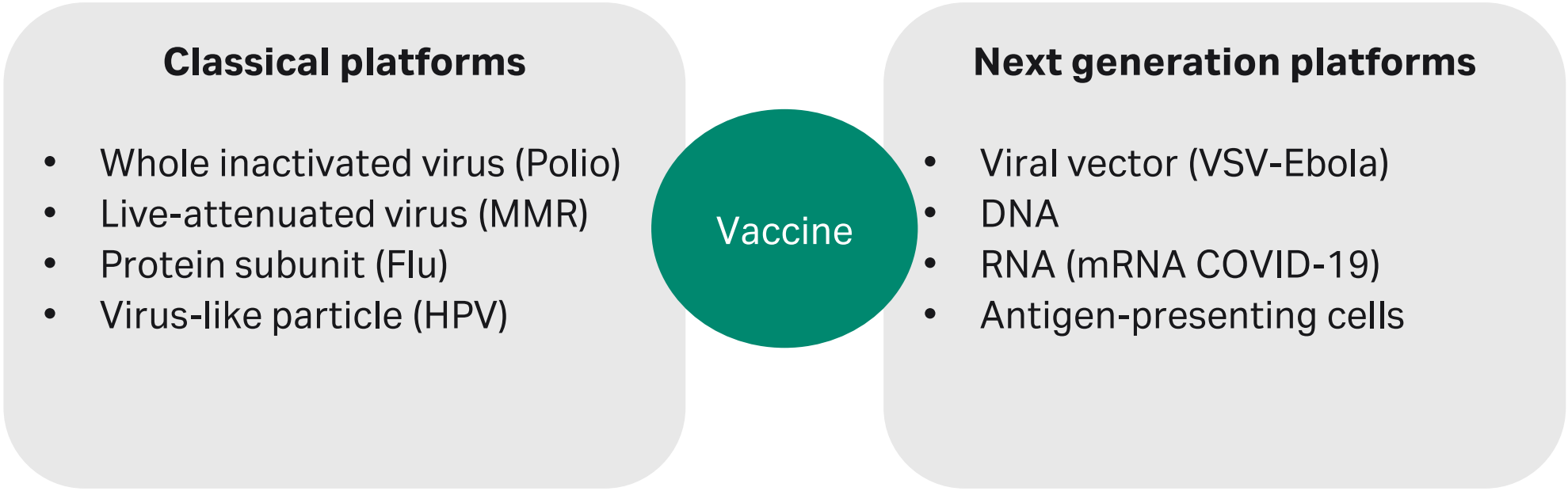
Introduction

- Vaccine equity means that vaccines should be allocated across all countries based on needs
- In 2021, the World Health Organisation (WHO) set the target for 70% global vaccination coverage by mid 2022
- This hasn't been achieved and was last reported at 52%
- High-income countries start vaccination on average two months earlier than low income countries (WHO 2023)
- To achieve 70%, low-income countries face an affordability issue. High-income countries need to increase their health spend by 0.8% on average to cover this, but for low-income countries it's 56.6%
- Achieving vaccine equity is complex, with countries needing to address many issues, such as manufacturing, procurement, distribution, education, and uptake



Data from: https://www.who.int/campaigns/vaccine-equity accessed 2023

Next generation vaccine platforms



Next generation vaccine platforms are focusing on innovations to speed up vaccine development and broad spectrum vaccines. These platforms could offer benefits such as being accessible, affordable, easy to store, transport, and administer and so they could help close the gap in vaccine equity

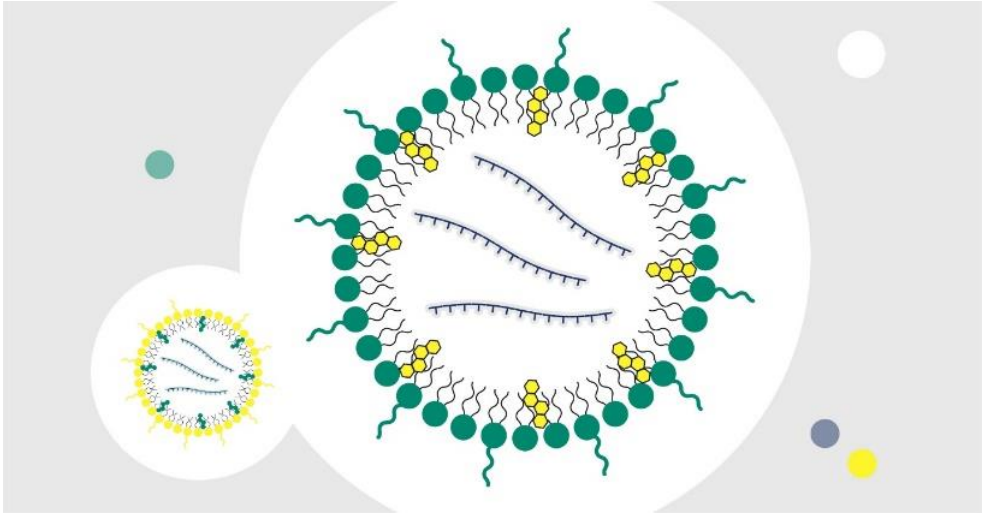
MMR – measles, mumps, rubella
HPV – human papillomavirus
VSV – vesicular stomatitis virus

How can mRNA help?

- Compared to traditional viral vector systems, mRNA systems are much faster because they don't require animal cells
- Changing parameters in cell-based manufacturing usually results in time delays, compared to mRNA manufacturing
- mRNA vaccines offer the potential to be completed in as little as five weeks[1]
- In comparison, viral vector systems can take around six to 36 months[2]
- The increased speed from discovery to delivery for mRNA vaccines can help reduce costs in process development

"Changing the equation"

- *mRNA vaccines are transformative. A powerful modality that allows universal vaccine infrastructure, thus bringing vaccine equity to developing countries.*
- *The mRNA vaccine technology transfer hub (created by the World Health Organization and other groups) seeks to empower low- and middle-income countries to produce their own vaccines instead of relying on other regions of the world.*



1. Saville M, Cramer JP, Downham M, et al. Delivering pandemic vaccines in 100 days—what will it take? *N Engl J Med*. 2022;387(2):e3
2. Rosa SS, Prazeres DMF, Azevedo AM, Marques MPC. mRNA vaccines manufacturing: challenges and bottlenecks. *Vaccine*. 2021;39(16):2190-2200

1

mRNA

mRNA therapy — from mass population to personalized

Pandemic response

- Few but large therapies to large populations
- Number of doses: one – two, yearly booster shots
- 10 L – 100 L IVT scale
- Production/year: < 1 kg – 300 kg
- Large manufacturing, localized, pandemic preparedness

Other infectious diseases and oncology

- Potentially many different therapies to medium to small populations
- Number of doses: one - life long
- 0.1 L – 10 L IVT scale
- Production/year: < 1 g – 50 kg
- Flexible manufacturing, multi product, and/or scaled out

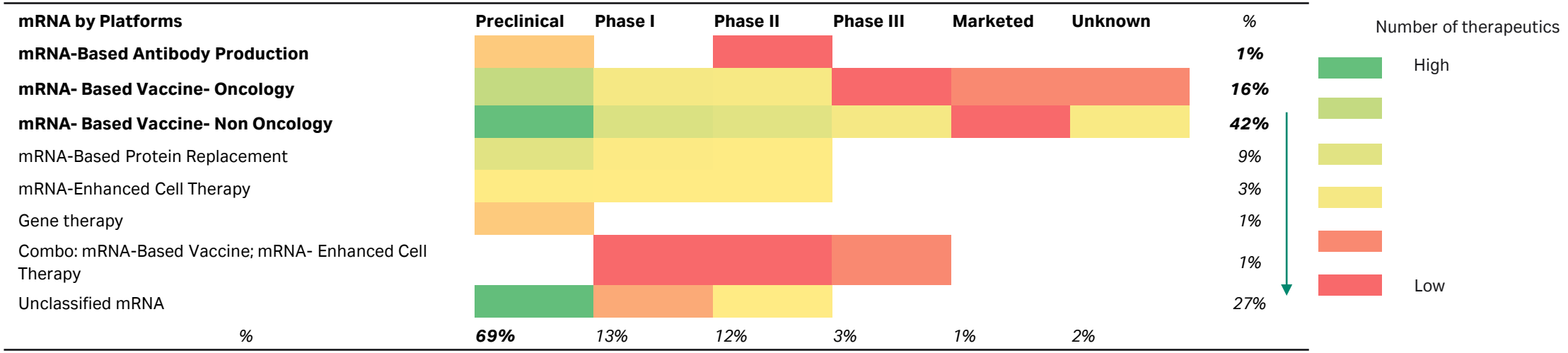
Personalized therapies

- Doses: µg – mg
- Number of doses: one – life long
- 10 mL - 50 mL IVT scale
- Production/year: < 1 g
- Collapsed workflow in micro factory

IVT – invitro transcription

Therapeutic promise

- With nearly 500 therapeutics in the pipeline, the mRNA landscape is evolving quickly
- Majority of mRNA therapeutics are in preclinical stage (69%)
- Increases in indication and therapy diversity including vaccine, gene editing, protein and antibody replacement, and *ex vivo and in vivo* cell applications
- Majority of mRNA drugs are vaccines for non-oncological indications (approximately 42%), followed by vaccines for oncological indications (16%) and protein replacement therapies (9%)



Data source: table and slide from Beacon 2022

mRNA manufacturing at scale: considerations and strategies

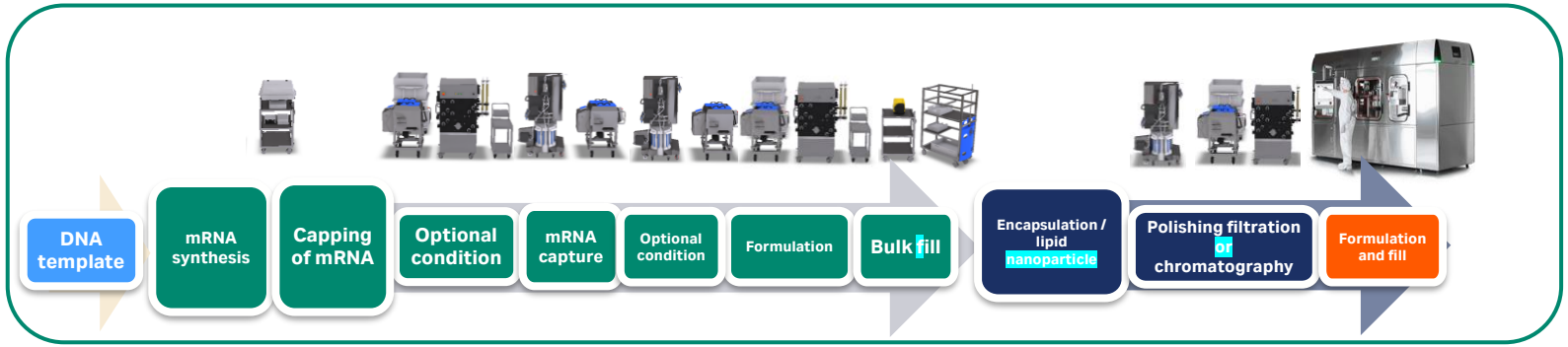
	DNA template	mRNA IVT	Purification	LNP formation	Filling
Considerations	• Quality and availability of template DNA • pDNA or synthetic DNA • Template design	• *In vitro* transcription (IVT) and capping efficiency • Capping strategy • Titer variations • Impurities • Scale	• Nonscalable methods • Capacity • Selectivity • Yield • Product stability • Scale	• Efficiency • Access to lipids • Solvents • Outsource or insource • Limited industry experience and expertise	• High dose volume • Multi-product • Often outsourced • Stability • Quality control
Strategies	– Insourced pDNA manufacturing	– Reagent sourcing and accessibility – Process control to reduce batch-to-batch variability	– Resins, membranes, and filters specifically developed for mRNA combined with traditional technologies – Flexible equipment	– Insourced encapsulation – Scale out to maintain quality with improved timeline – Access to lipid library	– Closed robotic filling equipment – Scale out or scale up filling to match dose output need – Insourced filling capacity

LNP – lipid nanoparticle
pDNA – plasmid DNA

Scale versus requirements

Large to mid-sized batch mRNA vaccine

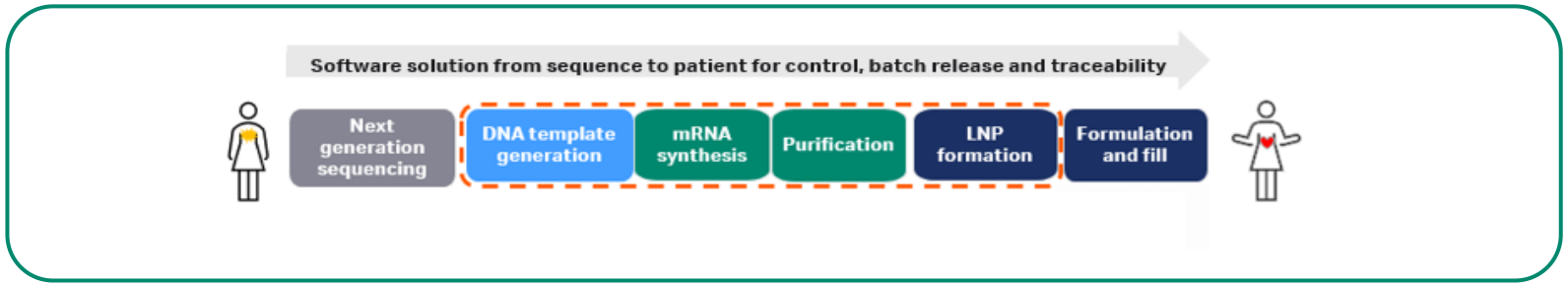
Needs:
- Yield
- Quality
- Capacity



Innovation in personalized scale will influence large-scale mRNA vaccines

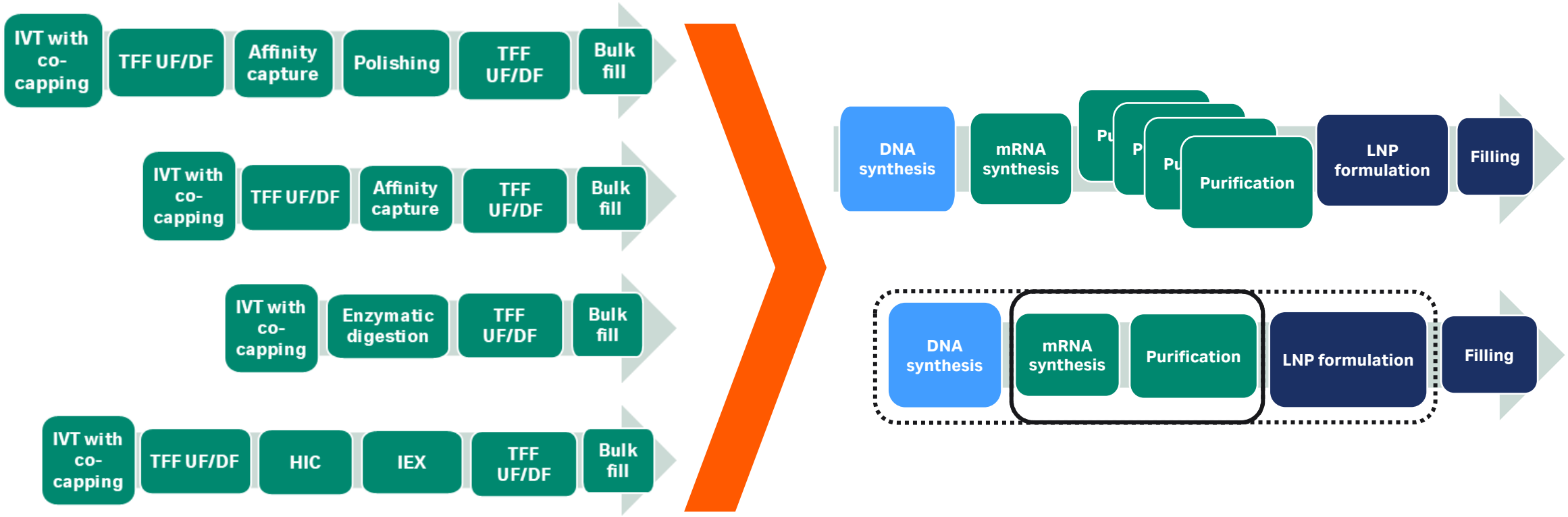
Small to mid-scale batch incl. personalized therapies

Needs:
- Integrated/automated
- Scale out
- COGs
- QC/patient tracking



COGs = cost of goods
QC = quality control

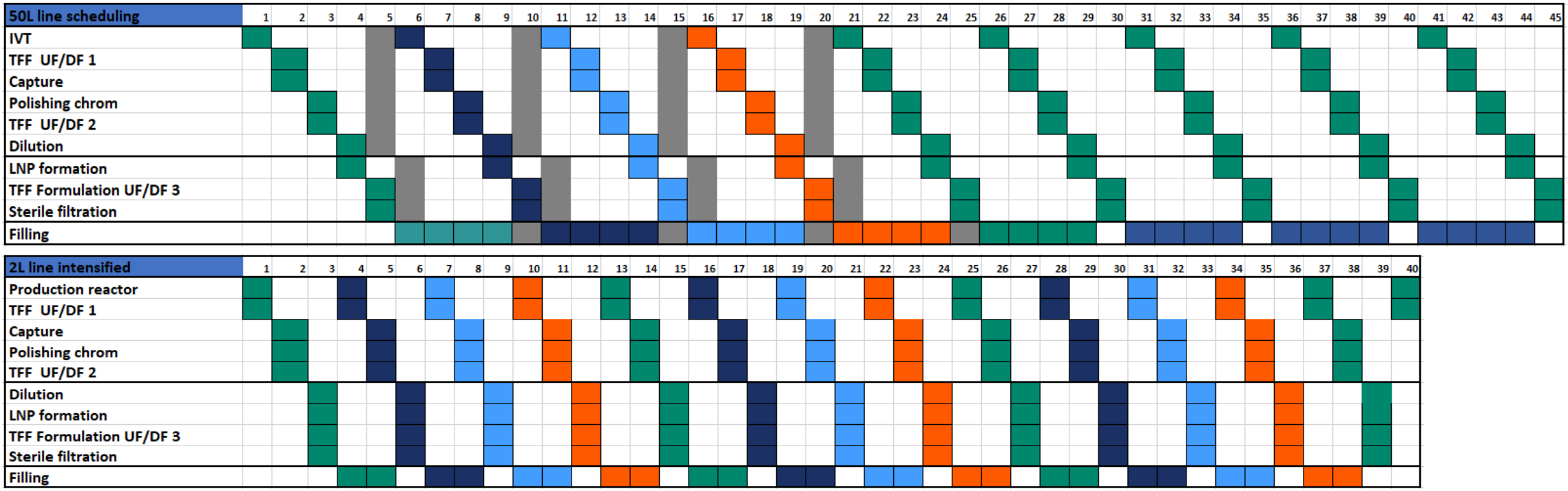
Process diversity in small scale



- mRNA processes today are diverse, highly dependent on the size of the construct, capping strategy, and IVT efficiency
- Different purification strategies may have to be considered
- Different drivers and strategies for handling screening phases and decreasing manufacturing scales
  - A flexible manufacturing platform with separate but connected unit ops
  - A printer or box setting with fully integrated unit operations

DF/UF – Ultrafiltration/Diafiltration
TFF = tangential flow filtration
IVT – Invitro Transcription
IEX – ion-exchange chromatography
HIC - Hydrophobic interaction chromatography

Manufacturing intensified scheduling



Multi-product and change-over

- Ballroom manufacturing and/or room segregation
- Closed systems to avoid cross-contamination
- Single-use reduces cleaning between batches or products

Modular biomanufacturing solutions for mRNA

Small and intensified

Example mRNA process

- IVT at 5 g/L titer
- 40% – 60% total recovery
- 70 batches/yr – 80 batches/yr with 80% facility utilization
- 4 g/batch – 5 g/batch

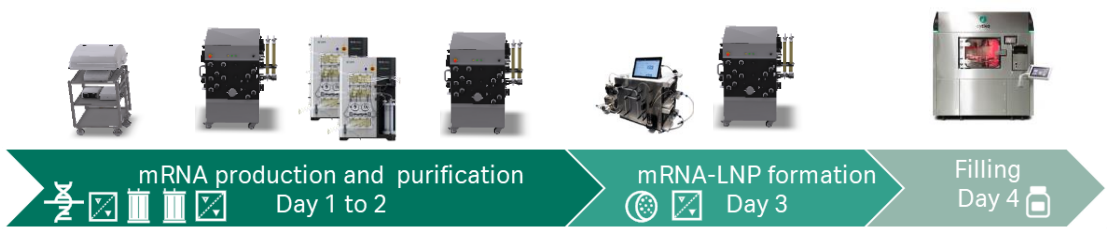
  250 g/yr – 300 g/yr

FlexFactory™ configurable manufacturing train

- Integrated manufacturing platform with flexible single-use equipment
- Industrial automation
- Consumables support
- Enabling services and training for faster time to engineering runs

Benefits of end-to end solution

- GMP requirements are the same regardless of scale
- Optimized manufacturing
  - Equipment utilization and re-use of unit ops
  - Footprint/manufacturing space
  - Consumables
- GMP ready with extended qualification
- Modular facility options available



GMP – good manufacturing process

Modular biomanufacturing solutions for mRNA

Mid to large scale: localized vaccine manufacturing

Example mRNA process

- IVT at 2 g/L – 5 g/L titer
- 40% – 60% total recovery
- 55 batches/yr with 80% facility utilization
- 40 g/batch – 60 g/batch

  2.2 kg/yr – 3.3 kg/yr

FlexFactory™ configurable manufacturing train

- Integrated manufacturing platform with flexible single-use equipment
- Industrial automation
- Consumables support
- Enabling services and training for faster time to engineering runs

KUBio™ manufacturing facility solution

- Designed for the 50 L mRNA FlexFactory™ manufacturing line, with optional prep and filling space
- Biosafety Level 1
- Expandable design for capacity increase

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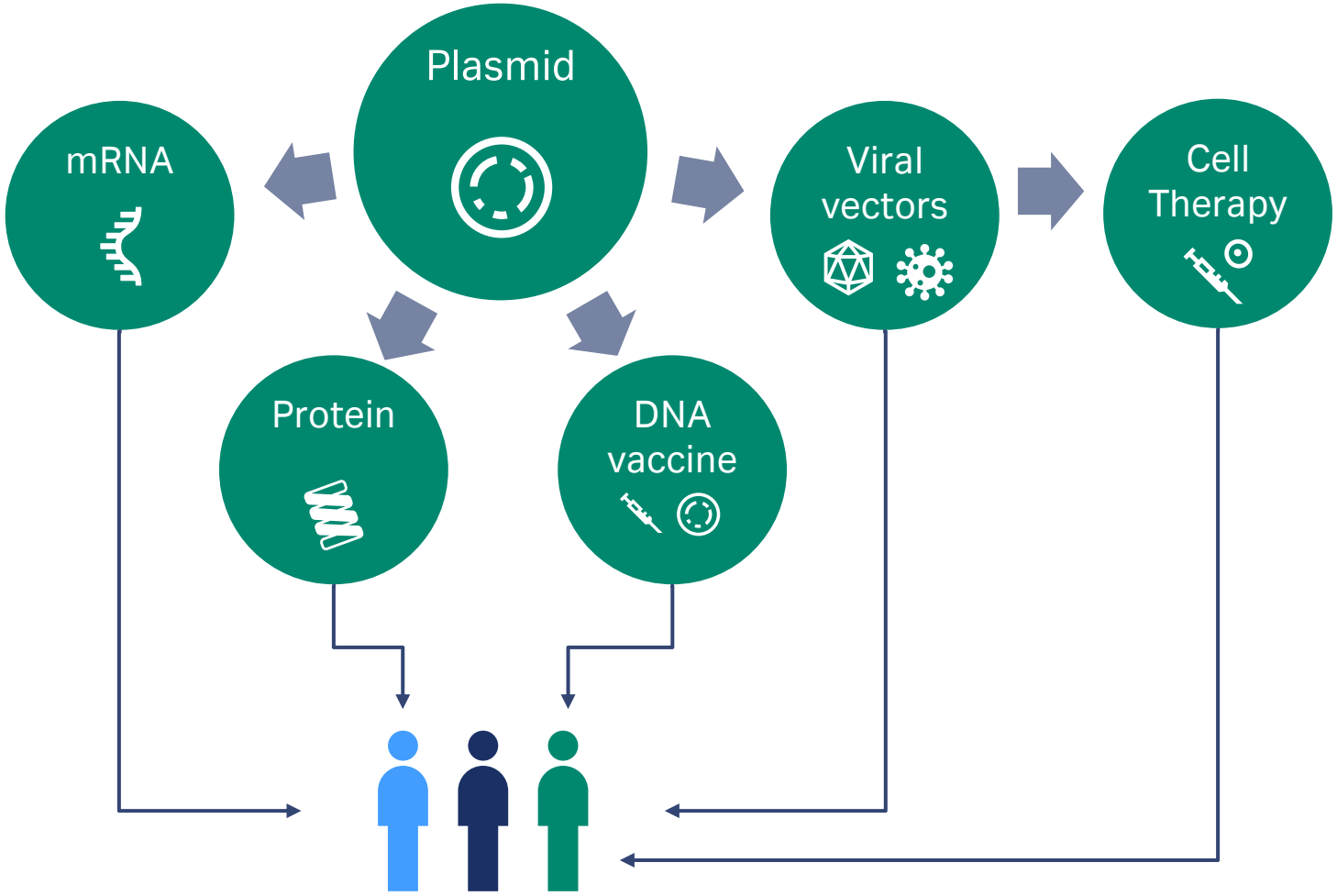
pDNA

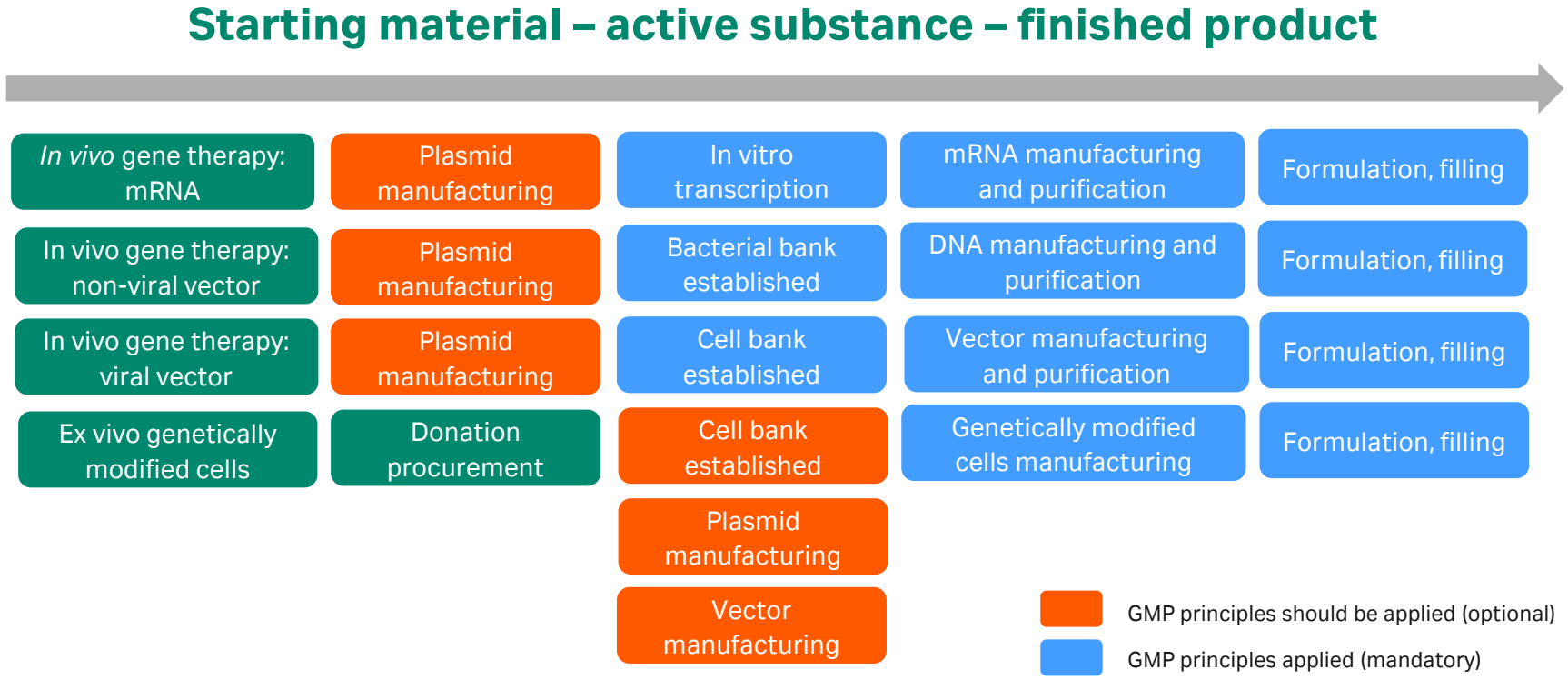
Plasmids – the start for many therapies

- In manufacturing of viral vectors for vector-based vaccines or Cell and gene therapy
- Direct as vectors for DNA vaccines/therapies
- Template for mRNA manufacturing
- Recombinant protein production



When is there a need for GMP plasmid DNA (pDNA)?

- High-quality pDNA is needed for manufacturing
- GMP certification might not be required, but it's mandatory to comply with GMP principles
- Example: In 2018, the presence of trace amounts of DNA fragments were identified in research-grade third-party supplied plasmid in an AAV manufacturing lot intended for clinical trials. FDA put in a temporary hold on the clinical trial. The company made the commitment to use GMP-S plasmid for all future production lots*



A table showing examples of where GMP or GMP principles apply in manufacturing, adapted from https://www.ema.europa.eu/en/documents/other/questions-answers-principles-gmp-manufacturing-starting-materials-biological-origin-used-transfer_en.pdf

AAV – adeno-associated virus
GMP – good manufacturing practice
GMP-S – GMP-Source®

* Sarepta Announces Clinical Hold Lifted for its Duchenne Muscular Dystrophy Micro-dystrophin Gene Therapy Program https://investorrelations.sarepta.com/news-releases/news-release-details/sarepta-announces-clinical-hold-lifted-its-duchenne-muscular

Plasmid GMP manufacturing

Primary objective

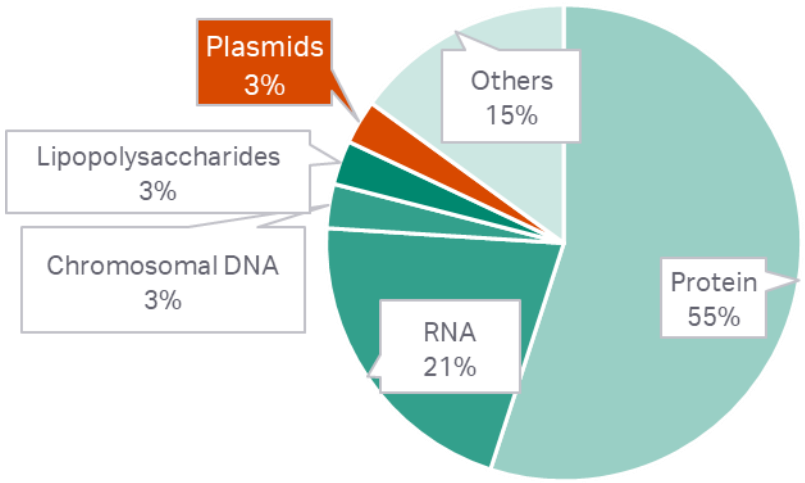
Manufacture the supercoiled plasmid of interest

Other objectives

- Meet output and quality needs
  - Different therapies have different needs
- Time to market
  - Ease of manufacturing
  - Ease of batch release
- Flexibility for multi-products

Challenges

- Different plasmids and applications may have different requirements
- Meet yield and purity goal
- Access to manufacturing capacity



Bacterial content

Data from Cytiva: 2022

Strategies

- Design the process to meet the scale and purity goals
- Modular and single-use, for flexibility with equipment and facility
- Integrated solutions for control, compliance, and efficient manufacturing

Optimization of pDNA manufacturing process

Primary manufacturing objective

Manufacture the super coiled plasmid of interest

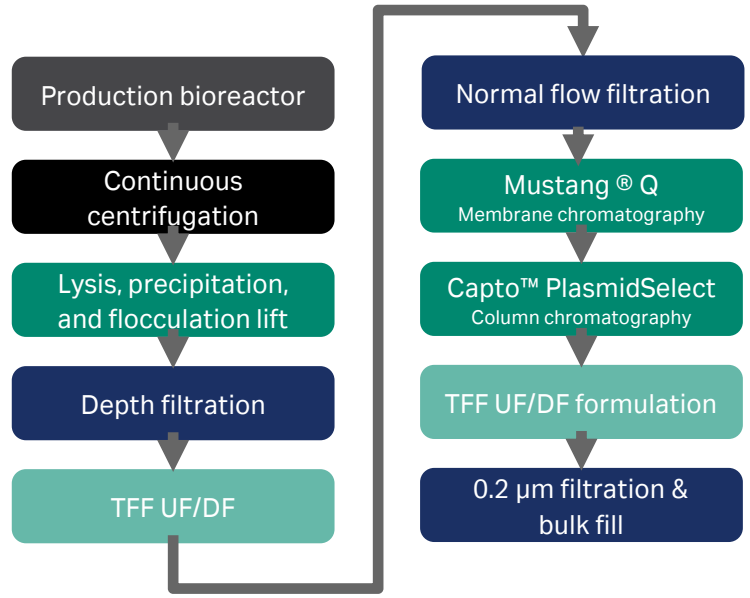
Other objectives

- Meet output and quality needs
- Flexibility for multi-products
- Economics

Optimization parameters

Process time	✓
Scalability and flexibility	✓
Increased process control	✓
Batch cost (OpEx)	✓
Footprint optimizing	✓
Environment – waste handling	✓

Two-step chromatography process



OpEx – operating expense

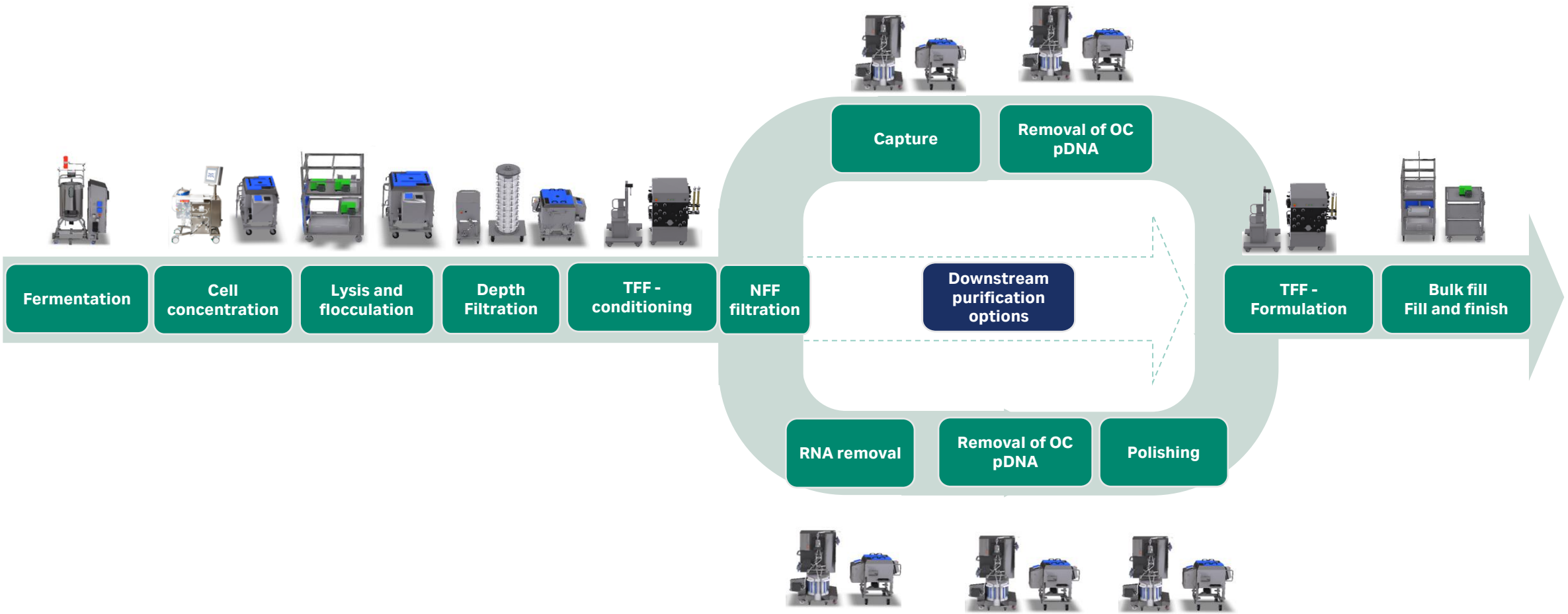
Upstream and midstream considerations

- pDNA is often a low-density fermentation
- Optimize the fermentation for titer and pDNA quality
- There are different options for cell concentration
- Lysis parameters are critical to maximize:
  - process efficiency for pDNA release and to facilitate clarification of the lysed material
  - removal of HCP, RNA, genomic DNA, and endotoxins

Set downstream up for success by optimizing the upstream and midstream

HCP –host cell protein

Two-step downstream pDNA purification process



NFF – normal flow filtration
OC – quality control

Modular biomanufacturing solutions for pDNA

50 L process example assumptions

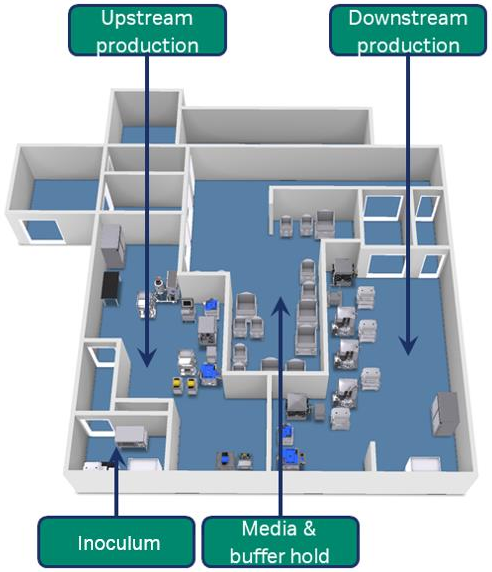
- *E. coli* low optical density (OD) fermentation
- 0.2 g/L titer @ 40L final volume
- 36% total recovery
- 48 batches/year with 80% facility utilization
- Approximately 2.9 g of plasmid/batch; and 0.14 kg/year

FlexFactory™ manufacturing line

- Integrated manufacturing platform with flexible single-use equipment
- Industrial automation
- Consumables support
- Enabling services and training – speed to engineering runs

KUBio™ box facility solution

- Designed for the 40 to 160 L plasmid FlexFactory™ manufacturing line
- Biosafety Level 1
- Expandable design for capacity increase



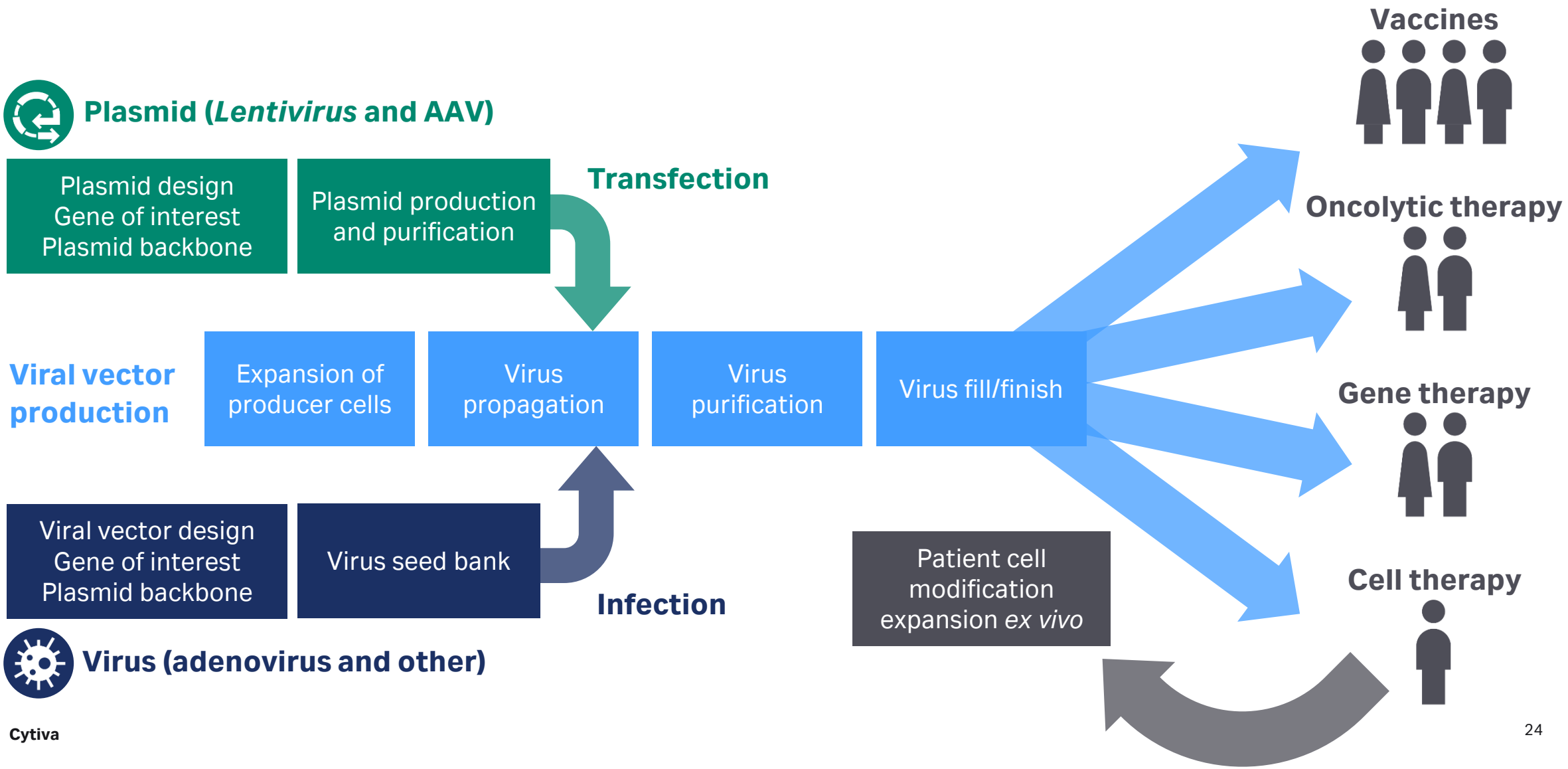
USP – upstream process
DSP – downstream process

3

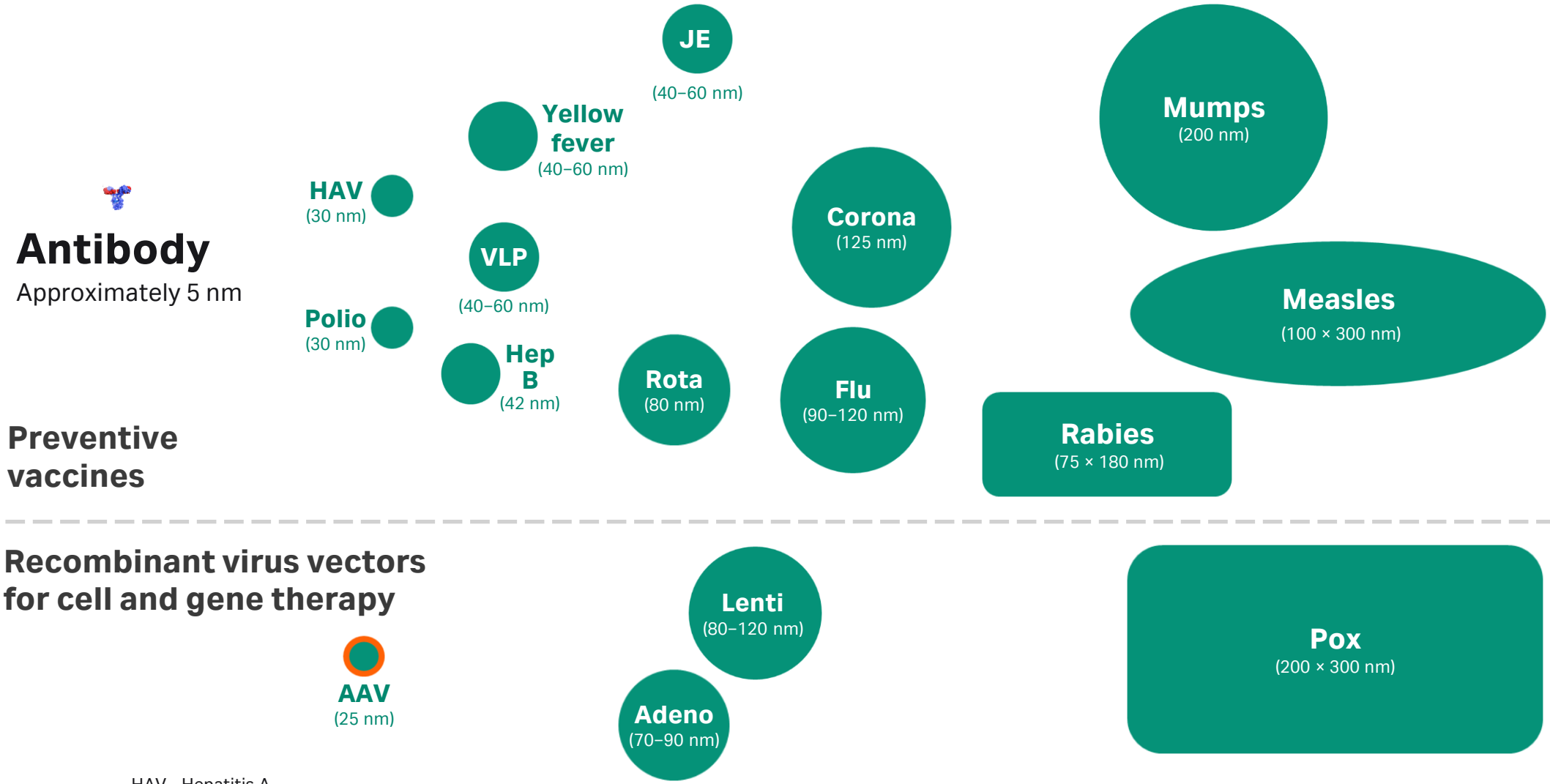
Viral vector

- AAV-Adeno

Viral vector production and clinical use



Sizes of common viruses



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HAV - Hepatitis A
Hep B – Hepatitis B
JE - Japanese encephalitis
VLP – virus-like particles

AAV is a widely used viral vector for gene therapy

Target organs determine selection of serotype

Tissue	Optimal serotype
CNS	AAV1, AAV2, AAV4, AAV5, AAV8, AAV9
Heart	AAV1, AAV8, AAV9
Kidney	AAV2
Liver	AAV7, AAV8, AAV9
Lung	AAV4, AAV5, AAV6, AAV9
Pancreas	AAV8
Photoreceptor cells	AAV2, AAV5, AAV8
RPE (retinal pigment epithelium)	AAV1, AAV2, AAV4, AAV5, AAV8
Skeletal muscle	AAV1, AAV6, AAV7, AAV8, AAV9

Engineered capsid variants are being developed for improved efficacy and tissue specificity

AAV = Adeno-associated virus

Top considerations for AAV manufacturing

AAV production

- Low proportion of full capsids in harvested material
- High levels of empty capsids reduces efficiency and performance in DSP

Harvest and filtration

- Lysis of cells to release virus
- High levels of HCP and hcDNA may reduce filtration capacity

Polishing

- Separation of full and empty capsids
- Optimize to maximize separation for each serotype
- Trade-off between viral genome recovery and full capsid percentage

Analysis

- Full and empty capsid ratio
- Critical for optimizing polishing step
- Accuracy depends on the method used

HCP = Host cell protein
hcDNA = Host cell DNA

Modular biomanufacturing solutions for AAV

200 L fed-batch process example

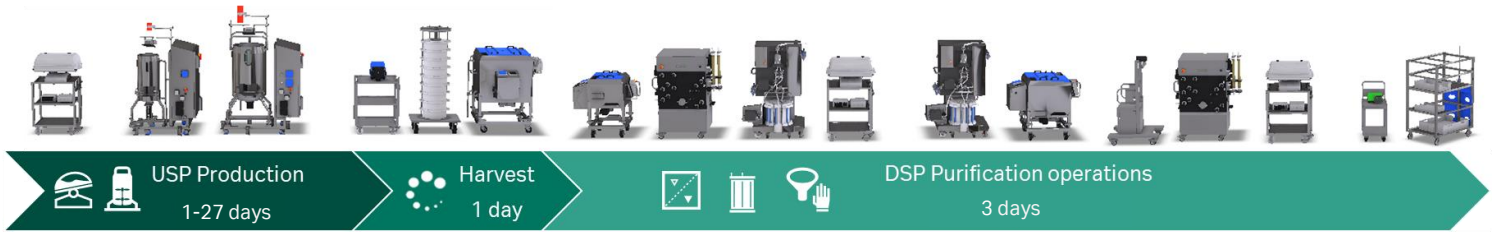
- Triple transfection of HEK293 producer cell line
- One upstream trains supported by one downstream train
- 1.0E+14 vp (viral particle)/L titer
- 36% total recovery
- 28 batches per year with 80% facility utilization
- 7.3E+15 purified vp/batch and 2.0E+17 purified vp/yr product produced

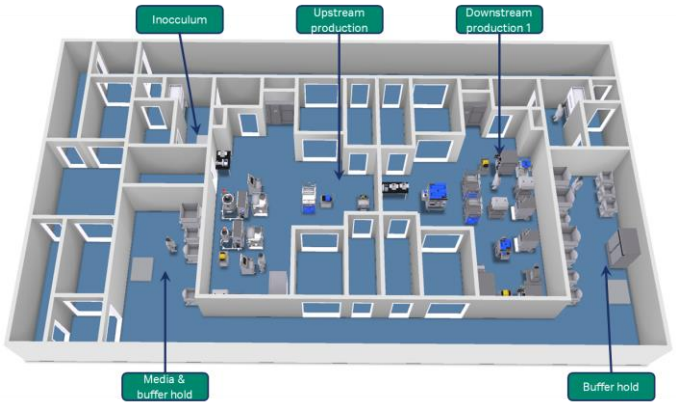
FlexFactory™ manufacturing line

- Integrated manufacturing platform with flexible single-use equipment
- Industrial automation
- Consumables
- Enabling services and training- speed to engineering runs

KUBio™ and KUBio™ box facility solution

- Designed for the 50-200 L AAV workflow*
- Biosafety Level 2
- Expandable design for capacity increase





Assumed process example, to be revised with customer's process details

** Larger scales supported by other designs*

4

Aseptic filling

Maximizing yield for multiple clients

- Goals:
  - Reduce risk
  - Maximize dosage yields
  - Serve multiple clients
- SA25 Aseptic Filling Workcells can fill all molecules and dosage types
- Scale out with standardized systems

Reduced risk via closed robotic workcells

- Remove human operator
- Remove quality risks
- Reduce product hazard pathway by using single-use flowpath
- Container / closure capable of -80°C

5

Manufacturability

How do you accelerate vaccine manufacturing?

Remove complexity

Future-proofing investments amid uncertainty

How do you know what's coming next?

What product will be manufactured?

What is the target therapy?

What is the manufacturing scale?

Access to qualified personnel and training?

Know the quality attributes.

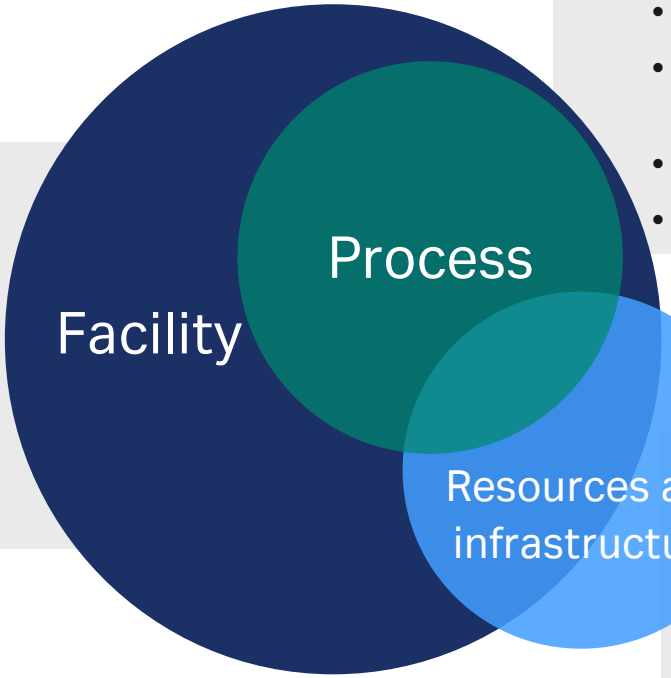
Attain manufacturability and scalability in process development.

Estimate your market size to scale manufacturing.

Make it easy to scale and expand.

The process is central to biomanufacturing

- A biomanufacturing enterprise includes process, facility, resources, and infrastructure
- These elements are integrated and influence each other
- Focus should be on understanding the product and its manufacturing process
- FlexFactory™ platforms and KUBio™ facilities are built around a process mass balance
- We offer process design services to support process understanding



- Layout
- HVAC and M/E/P installations
- Clean utility generation
- Waste treatment
- BMS and EMS

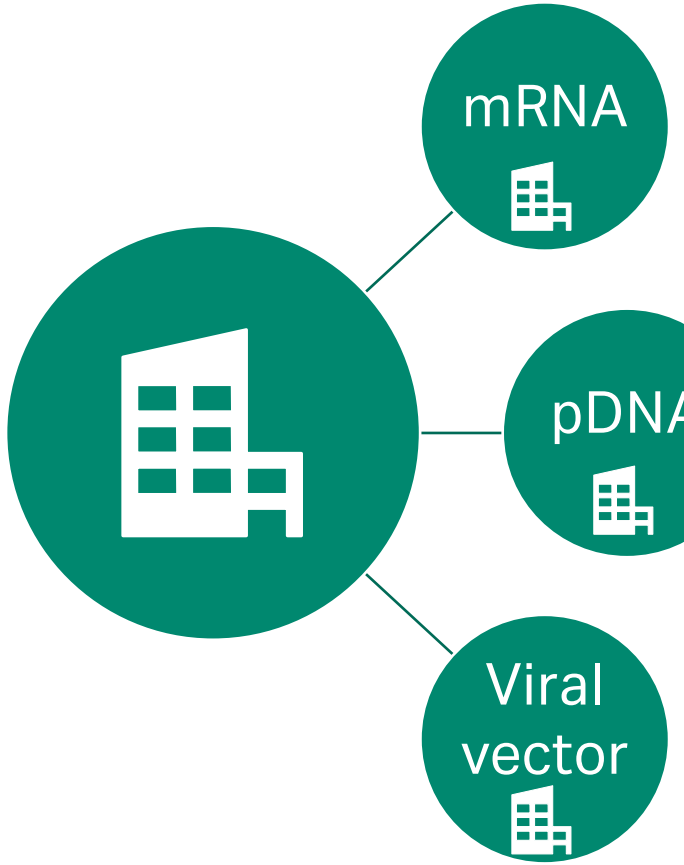
- Unit operations
- Equipment
- Process control system
- Input materials
- Output and yield

- People
- Warehouse
- QC or QA
- Manufacturing operations, management systems, and digital
- Regulatory, policies, and procedures

Heating, ventilation, and air conditioning
M/E/P - mechanical, electrical, and plumbing
BMS- building management system
EMS- environmental management system
QC – quality control
QA – quality assurance

Vaccine facility for multiple modalities

Centralization considerations



- Enzymatic reaction
- Sensitive to RNases
- Solvents in LNP formation

- Microbial process
- Endotoxins

- Mammalian cell culture as starting point
- Viral product, BSL2

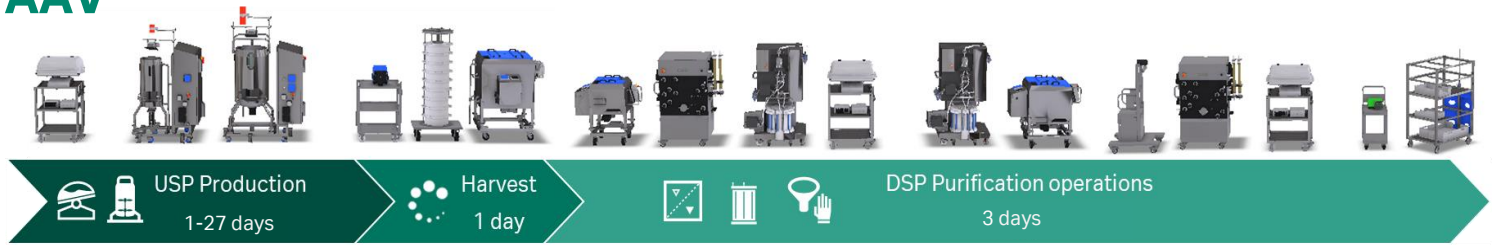
BSL2 – biosafety level 2

Is aggregation possible?

mRNA

pDNA

AAV



- Process equipment may be similar, but with different processes and various clean room requirements
- The following will need to be considered for modality workflows and their associated manufacturing steps
  - What needs to be segregated?
  - What can be shared?
  - What are the regulatory issues associated with this?

Buy or make buffers for the pDNA process?

Considerations

- Existing capabilities
- Volumes
- Consumables
- Warehouse/storage
- Labor
- QC and release

Cytiva solution

Buy:

- All buffers in the pDNA process are available through the Hyclone™ process liquids and buffers offering

Make:

- Mixers
- In-line dilution systems
- Bins
- Filters and tube sets

With intensification digitalization is essential

Data management

- Short processing time allows for many batches
- Chain of custody/identity and batch release at personalized scale

"..we then have to release that product and doing so in automated fashion is a value add.." "..for us, it's super relevant because we have to do it so many times, one for each patient.."

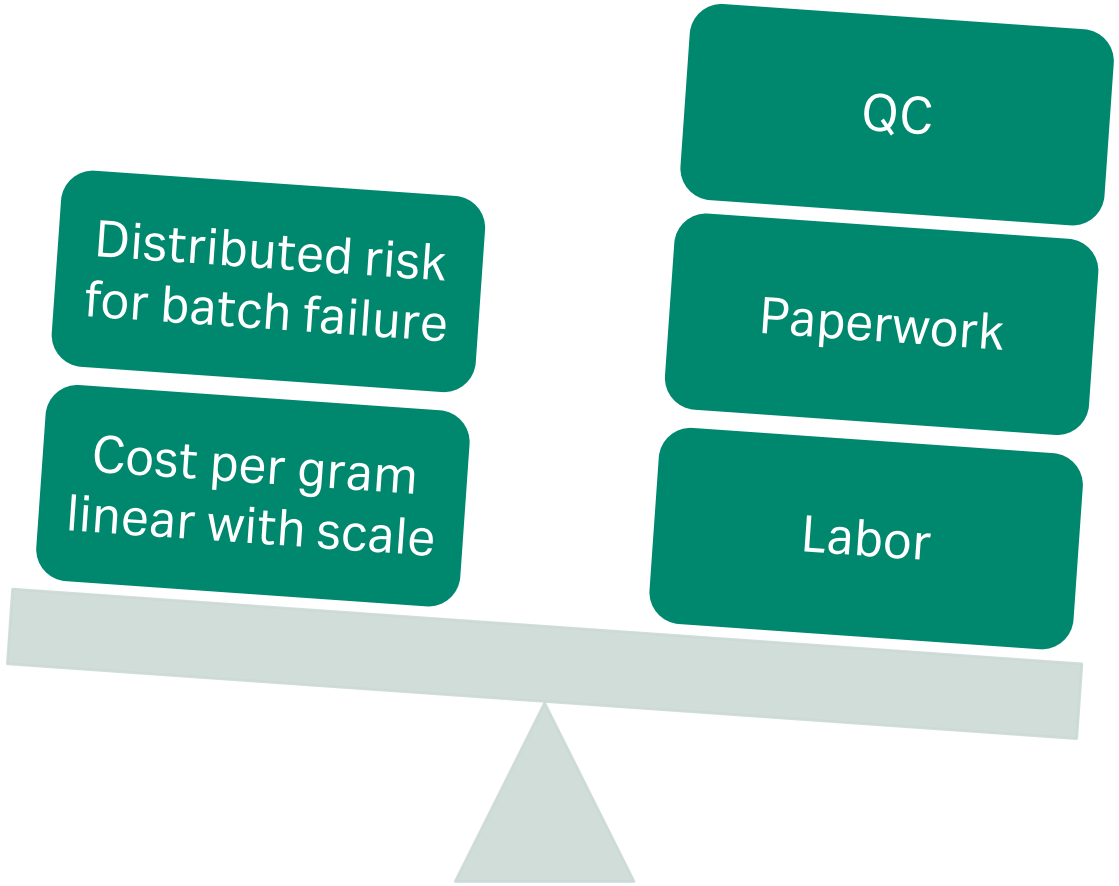
Risk of contamination

- mRNA is sensitive to contamination; autoclaving paper records in and out of the manufacturing suites is time consuming
- *"40 batches/week with paper batch records is autoclaved to reduce the contamination risk. It is A LOT of paper...."*

Manual entries and process steps

- Manual entries or transcription of paper-based data need 4-eye verification when being made digital
- Example: One mRNA batch has approximately 200 consumables items that needs to be logged in the batch record
- FDA requires electronic submissions

Smaller, intensified batches are de-risking manufacturing but shifting the burden to QC and paperwork



Delaying

- Time to revenue
- Time to market
- Time to therapy

Process intensification is enabled through smaller equipment but increasing the documentation burden

Drivers for a digitalized manufacturing

Additional annual throughput and increased speed

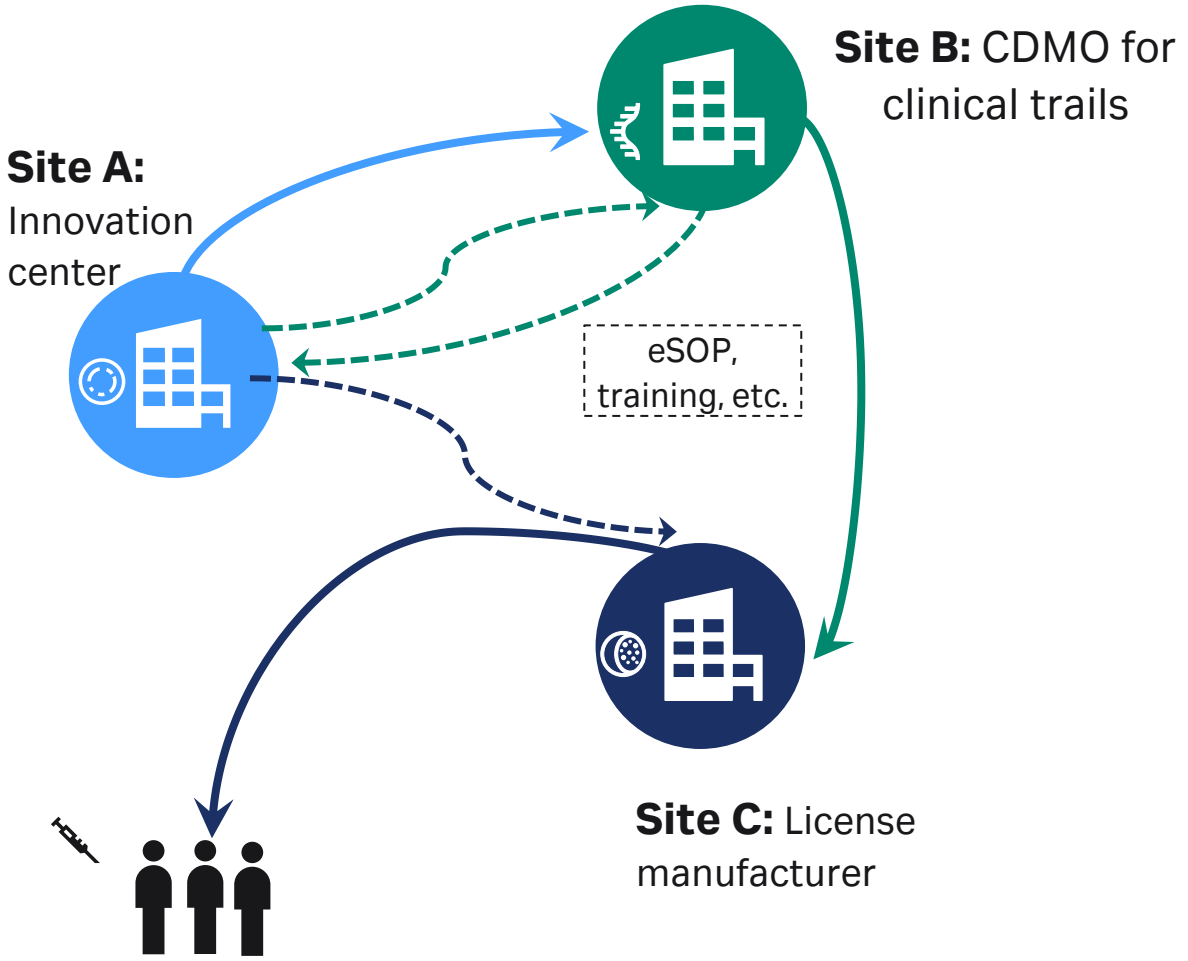
Increased quality - less errors in manufacturing, fewer deviation and ease of batch release

Drives down manufacturing costs - labor reduction being primary driver

"FDA is going to make everybody go electronic. We're going to see the disappearance of paper and that'll be great"

Digital solutions enables ease of process transfer and training

- Same solution used in pre-GMP as in GMP allows for easy transfer of electronic protocols
- Start working on manufacturing procedures during the early phases
- Potential to train and educate workforce



eSOP –electronic standard operating procedure
CDMO – Contract Development & Manufacturing Organization

The heart of science is measurement

QC procedures need to be specific to your manufacturing goals

Identity	• Sequence confirmation	• Next-generation sequencing (NGS) /Sanger sequencing/reverse transcriptase - PCR
Content	• RNA content	• RT-qPCR, RT-dPCR, ultraviolet spectroscopy
Integrity	• Intact mRNA vs fragment mRNA • 5′ Cap • 3′ poly A tail • mRNA integrity	• Capillary gel electrophoresis • IP-RP-HPLC • RP-HPLC • Gel electrophoresis
Purity	• Product-related impurities, dsRNA • Residual DNA template	• Immunoblot • qPCR
Safety	• Endotoxin • Bioburden • Sterility	
Other	• Appearance • pH	

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PCR – polymerase chain reaction
RT-qPCR – Quantitative reverse transcription PCR
RT-dPCR – Reverse transcription PCR
IP-RP-HPLC –ion-pair reversed-phase high performance liquid chromatography

Adapted from:
"Analytical Procedures for mRNA Vaccine Quality", Accessed 2022-MAY-30, https://go.usp.org/mRNAVaccineQuality

Addressing the challenges of multi-modality manufacturing

Futureproof by considering manufacturing at all stages and scales

Consider flexibility with modular platforms and manufacturing standardization

Ensure reproducibility through digitalization, automation, and contamination control

Establish control through quality processes relevant to your manufacturing

Thank you

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