cGMP virus manufacture and evolving release tests

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Advanced Therapy Medicinal Products:
from Promise to Reality
Regulatory path for translation of research
to commercial medicinal products
Brighton, United Kingdom

University of Oxford
Manufacturing for First in Man Clinical Trials

TAC History (Monoclonal Antibodies)

•1995 The University of Oxford has new GMP facility for the production of Investigational Medicinal Products (IMPs) to produce monoclonal antibodies and related biologics.

•2004 First academic facility to obtain a MHRA Manufacturing Authorisation for IMPs post EUCTD.

•TAC products have supported more than 5,000 patients in clinical trials worldwide.

•Star product Alemtuzumab estimated market product in peak year 2016 is $0.5 -$2 BILLION!

•2-3 other antibodies still in phase III clinical trials -with billion dollar plus sales potential

TAC to CBF Transition (Viral vectored Vaccines and Therapies)

•Nov 2005

Decision to move to manufacture Viral Vectors and transfer to NDM Jenner Institute

•2006

Building work & validation for change to manufacture Gene Therapy products

Update of MHRA Manufacturing Authorisation

•2007

Feb first product filled for clinical use

Oct 2007 first volunteer immunised

•Today

10 batches vaccines in phase I/II clinical trials worldwide.

2 manufactured and awaiting QC / QP release, others in pipeline

vectored vaccines are where monoclonals were 15 years ago

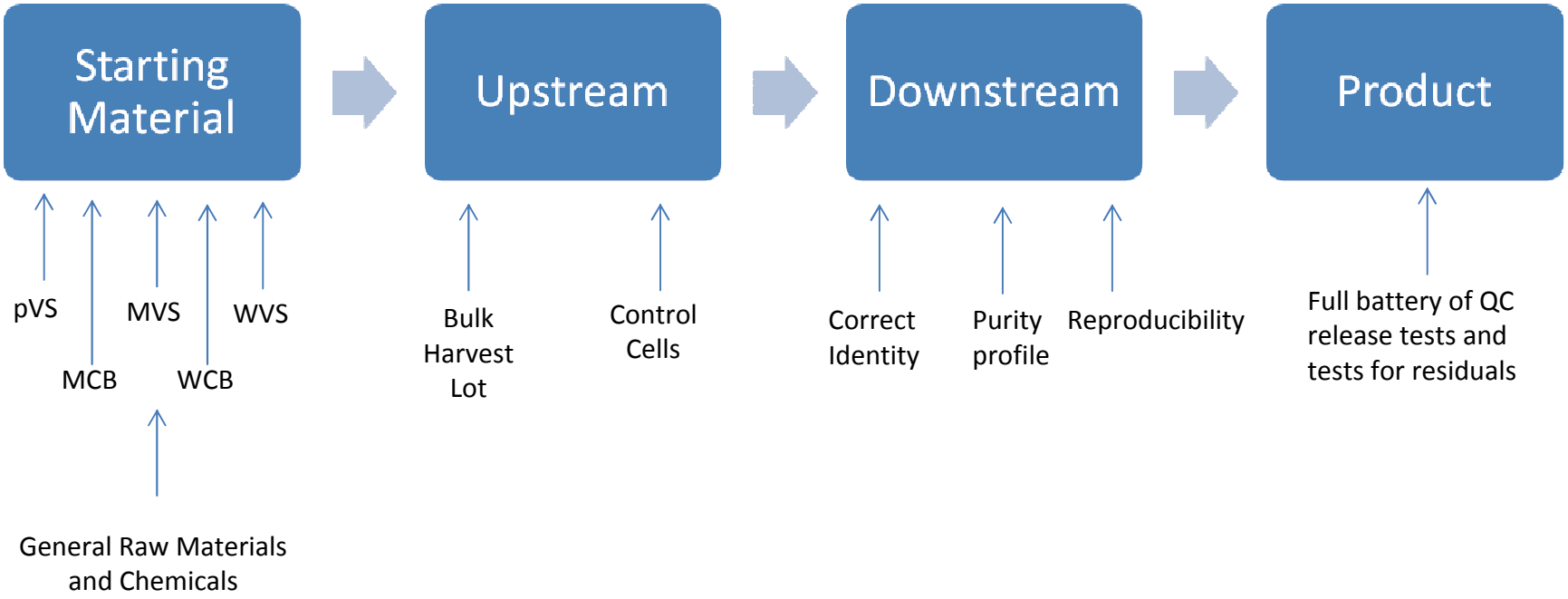
Questions

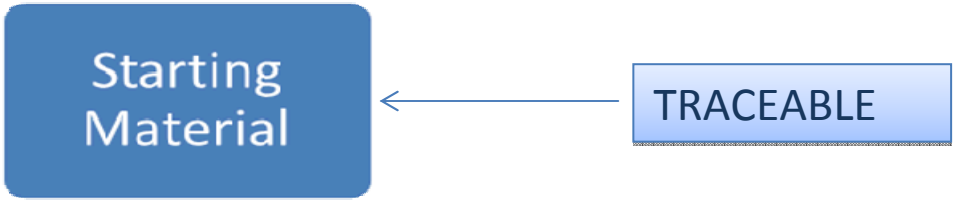
- Why are we testing products? – to ensure patient safety
- Why have a monograph when there are no licenced products? – guidance but what about replication competent versus replication incompetent - IMPs
- Inconsistencies amongst Competent Authorities

Advanced Therapy-

Viral Vectors, Cell Therapies

Differences/consistent approach required?





VIRUS			
Where was it isolated?	Primary isolate		
	Molecular clone		
	Synthetic DNA		
Where has it been grown?	Cells	Source	
		Labs	
		Tested	
		Traceable	
	Reagents	FBS	Gamma Irradiated
			Tested
		Trypsin	animal
			recombinant
		other supplements	
Other virus being grown concurrently?	Academic		
	Pharma		
	Biotech		

How can we ensure patient safety?

Generate Primary Virus Stock

DNA or Synthetic Virus – what are the risks?

↓

Fully tested cell bank – risks mitigated

↓

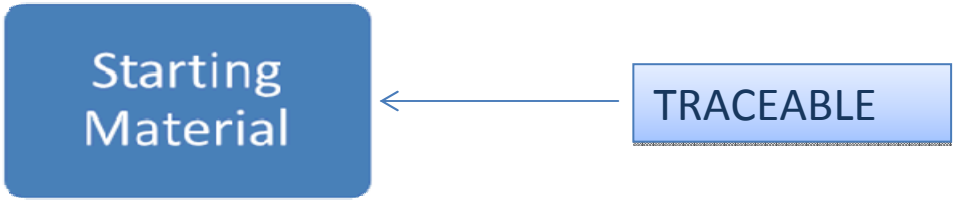
Recombinant dissociation - risks mitigated

↓

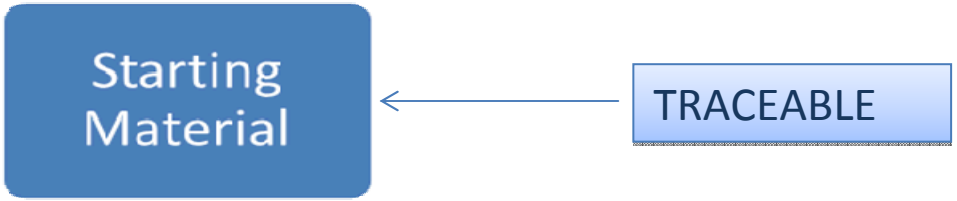
Expansion fully documented and qualified / validated
FBS or chemically defined media – risks mitigated

↓

Risks left from operators/ environments



CELLS			
Source?	Primary cells	Species	
	Cell line	Human vs animal	Organ
	Previous use	Existing cell substrate	
Supplements?	Recombinant	Source	
		Labs	
		Tested	
		Traceable	
	Animal derived	FBS	Gamma Irradiated
			Tested
		Trypsin	animal
			recombinant
		other supplements	
Other factors?	Scale		
	Reliability of supply		
	Shelf life		

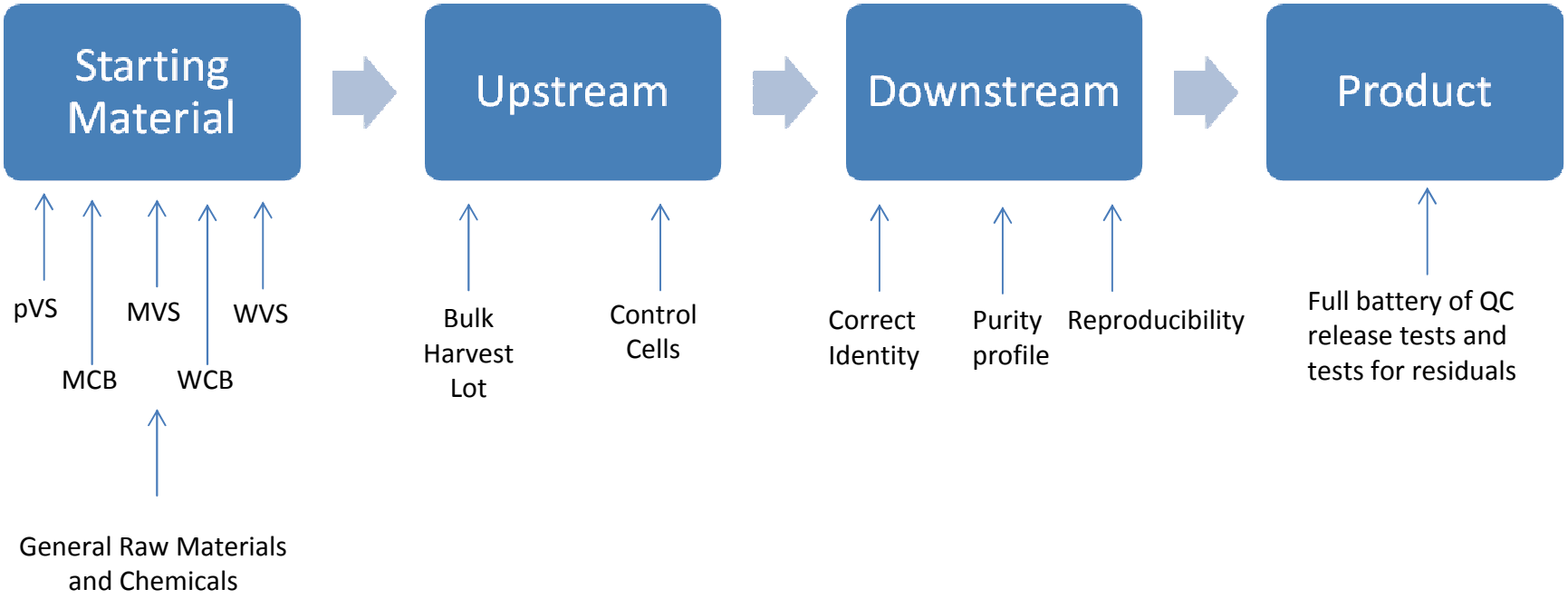


REAGENTS			
Media?	Chemically defined		
	Synthetic		
	Off the shelf		
Media?	Cells	Source	
		Labs	
		Tested	
		Traceable	
	Reagents	FBS	Gamma Irradiated
			Tested
		Trypsin	animal
			recombinant
		other supplements	
	Supply		
	Shelf life		
	Known hazards		

Advanced Therapy-

Viral Vectors, Cell Therapies

Differences/consistent approach required?



Adenovirus Safety Testing - CELLS

Assay Type	Complementing Cell MCB	Complementing Cell WCB	Control Cell Bank (cells at limit of In vitro age - EPC)
Identity			
Isoenzyme	x	x	x
DNA Fingerprinting	x	x	x
Microbiology			
Sterility - Harmonised USP/EP/JP protocol	x	x	x
Sterility Qualification - USP/EP/JP protocol	x	x	x
Mycoplasma EP (including assay qualification) - issues with Vero cell culture/adenovirus	x	x	x
Adventitious Virus			
In vitro (28 day) - MRC-5/Vero/HeLa detector cell lines	x	x	x
In vivo - adult & suckling mice, embryonated eggs, guinea pigs	x		
In vivo - adult & suckling mice, embryonated eggs			x
Bovine *In vitro* - 9 CFR or CVMP	x		
Porcine *In vitro* - 9 CFR	x		
Retrovirus			
Transmission EM Profile of 200 Cells	x		x
F-PERT Assay for Reverse Transcriptase	x	?x	x
Co-cultivation of cells with F-PERT end point - required before licensing, but may be deferred unless is needed to resolve PERT result.			x

Adenovirus Safety Testing - CELLS

Assay Type	Complementing Cell MCB	Complementing Cell WCB	Control Cell Bank (cells at limit of In vitro age - EPC)
Species Specific Virus / Pathogens			?
Hep A Q-PCR	x		
Hep B Q-PCR	x		
Hep C Q-PCR	x		
HIV 1 & 2 Q-PCR	x		
HTLV I & II Q-PCR	x		
B19 Parvovirus Q-PCR	x		
EBV Q-PCR	x		
CMV Q-PCR	x		
HHV 6 Q-PCR	x		
HHV 7 Q-PCR	x		
HHV 8 Q-PCR	x		
SV 40 Q-PCR	x		
AAV Q-PCR	x		
And any other identifiable risk - tumorigenicity	? x		x

Adenovirus Safety Testing - VIRUS

Assay Type	Master Virus Seed Stock	Working Virus Seed stock	Adenovirus Bulk Harvest	Bulk Purified Adenovirus	Clinical Batch
Identity					
Vector identity - PCR / immunological	x	x	x	x	
Microbiology					
Sterility & Bacteriostasis – Eur.Pharm.	x	x	x	x	x
Mycoplasma & Mycoplasmastasis – EP	x	x	x		
Mycobacterium – EP	x	x	x		
Adventitious Virus					
In vitro (28 day) - MRC-5/Vero/HeLa detector cell lines	x		x		
In vitro assay - cytotoxicity & interference studies on r-adenovirus – effectiveness of neutralising antisera	x		x		
In vivo - adult & suckling mice, embryonated eggs, guinea pigs	x		1		
In vivo toxicity studies on r-adenovirus – effectiveness of neutralising antisera	x		1		
Bovine *In vitro* - 9 CFR Guidelines + Cytotoxicity	x				
Porcine *In vitro* - 9 CFR Guidelines+ Cytotoxicity	x				
Retrovirus					
F-PERT Assay for Reverse Transcriptase	x				

1 – The Eur.Pharm. & latest FDA Vaccine Substrate guideline, no longer requires *in vivo* batch testing if the MCB & MVSS are tested.

Adenovirus Safety Testing - VIRUS

Assay Type	Master Virus Seed Stock	Working Virus Seed stock	Adenovirus Bulk Harvest	Bulk Purified Adenovirus	Clinical Batch
Species Specific Virus / Pathogens					
Hep A Q-PCR	X				
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HIV 1 & 2 Q-PCR	X				
HTLV I & II Q-PCR	X				
B19 Parvovirus Q-PCR	X				
EBV Q-PCR	X				
CMV Q-PCR	X				
HHV 6 Q-PCR	X				
HHV 7 Q-PCR	X				
HHV 8 Q-PCR	X				
SV 40 Q-PCR	X				
AAV	X				
And for any other identifiable viral risk	?				

Adenovirus Safety Testing - VIRUS

Assay Type	Master Virus Seed Stock	Working Virus Seed stock	Adenovirus Bulk Harvest	Bulk Purified Adenovirus	Clinical Batch
Adenovirus Specific					
RCA on A549 Cells (replication defective only)	x			x	
Infectivity / Particle Ratio	x	x		x	x
Infectious Adenovirus Titre	x	x		x	x
Genetic Stability Tests					
Sequencing - whole virus required				x one time only	
Genetic integrity by restriction analysis	x	x		x one time only	
Additional Batch Release Assays					
Residual Host cell DNA				x	
Residual Host cell DNA fragment length				x	
Residual Bovine Serum Albumin (if FCS used?)				x	
Residual "Benzonase"				x	
Residual Host cell protein				x	
Identity / Purity– SDS PAGE/Immunological				x	x
Bio-potency ?				x	x
Abnormal Toxicity/General Safety					x
LAL / Pyrogens				x	x
pH					x

Adenovirus Safety Testing - VIRUS

Assay Type	Master Virus Seed Stock	Working Virus Seed stock	Adenovirus Bulk Harvest	Bulk Purified Adenovirus	Clinical Batch
Additional Batch Release Assays					
Osmolality					x
Aggregates					x
Virus concentration					x
Infectivity					x
Particle : Infectivity ratio					x
Biological Activity					x
Replication competent adenoviruses				x	
Extractable volume					x
Thermal stability					x

Risks left from operators/ environments

- Should we not design a testing package based upon risk and not on documented Ph. Eur.
- Emphasis of testing should be on viruses that can be amplified in the cell substrates and systems used in the downstream process
- Ignore bovine, porcine viruses if using animal free systems
- Why repeat testing for viruses that cannot be amplified
- What are the risks from contaminating viral DNA: porcine circovirus

Are the risks the same for IMPs as opposed to marketed products?

- Advanced therapies very novel, often testing to prove or disprove a hypothesis, i.e. proof of concept trial – *no intention of being marketed*

- Clinical status of trial subjects

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Directive 2009/120/EC

This Directive was published 14th September 2009 and amends Directive 2001/83/EC relating to medicinal products for human use as regards advanced therapy medicinal products.

2.1 Gene Therapy Medicinal Product -Gene therapy product means a biological product which has the following characteristics:–

(a) it contains an active substance which contains or consists of a recombinant nucleic acid used in or administered to human beings with a view to regulating, repairing, adding or deleting a genetic sequence

(b) its therapeutic, prophylactic or diagnostic effects relates directly to the recombinant nucleic acid sequence it contains, or to the product of genetic expression of this sequence

Gene therapy medicinal products shall not include vaccines against infectious diseases