ATMP manufacture from an academic perspective

- Start development with the end in mind
- Good manufacturing practice
- Clinical trial application
- Plan ahead

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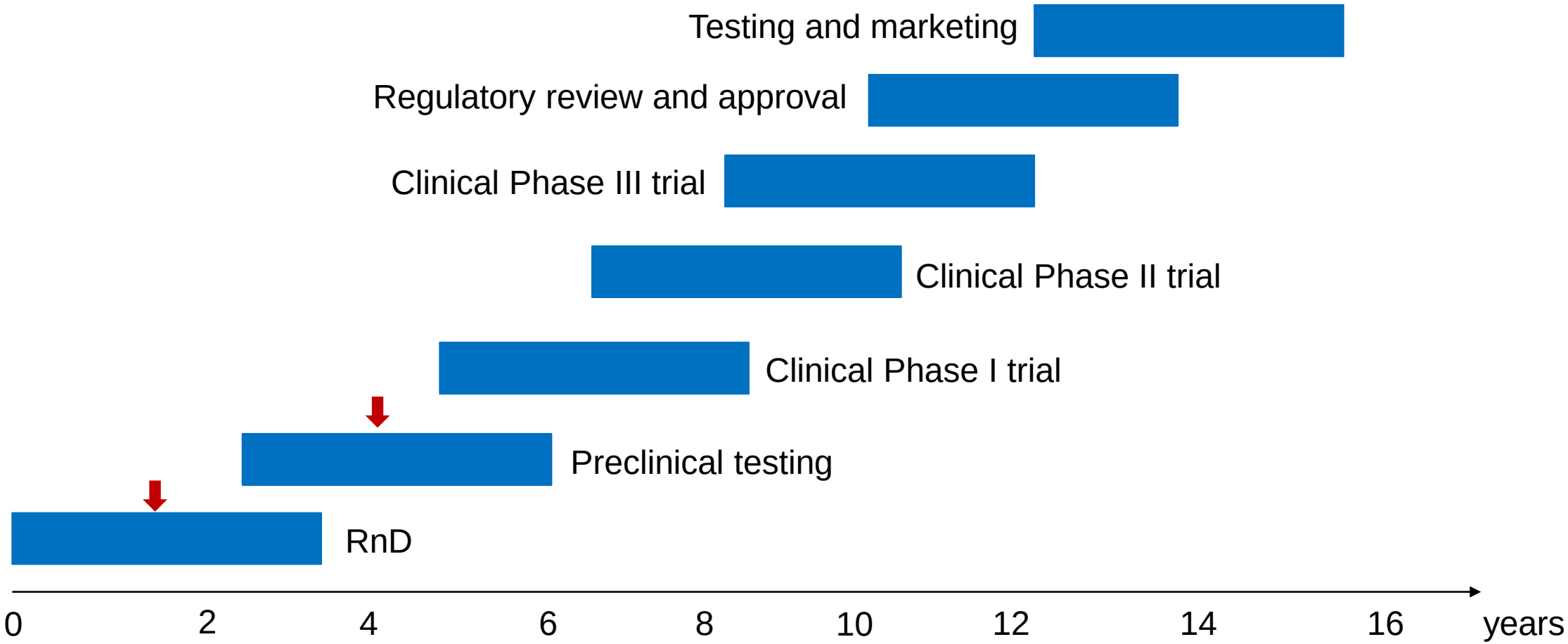
I have developed a medicine!!!

.....well.....

Timeline drug development



What is needed to go from test tube to candidate drug

- Clinical trial application
  - Investigators medicinal product dossier (IMPD)
  - Investigator´s Broschure (IB)
  - Clinical Trial Protocol (CTP)

- Adaptation to Good **manufacturing** practice (GMP)

- Ethics applications
  - Donor information and agreements
  - Patient information and agreements

Guidelines and directives to follow; but not always hands-on and specific
https://www.ema.europa.eu/en/human-medicines-regulatory-information

Start development with the end in mind

Some important parameters:

- Safety
- Effect
- Dosing
- Process
- Product
- Stability

Start development with the end in mind

- Safety first
- Effect- what do we want to achieve in the patients
- Dosing- amount and dosage form
- Process- same degree of safety and effect every time
- Product- same product every time
- Stability- for how long and how can the product be stored

Safety first

How do we guarantee that the end product is free from risks?

- Risk assessments
- Donor screening, e.g. HIV-1 and HIV-2, Hepatitis B and C, Syphilis, HTLV 1 and HTLV 2
- Additional testing of starting material e.g. screening for genetic aberrations
- Manufacturing process and raw materials
- Operators
- Clean room monitoring
- Testing of manufactured product
- Adverse effects, short term and long term

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Effect- what do we want to achieve in the patients

- How do we know that the drug actually works
  - *In vitro* studies
  - Data from animal models?
  - Human data?
- Potency assay
  - measure surrogate markers of clinical effect
  - but also to demonstrate that the manufactured drug is potent
  - and to detect changes in manufacturing process leading to a less potent drug

➢ e.g. bone differentiation, immunesuppression

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Dosing- amount and dosage form

- How much is safe to give
  - Data from animals relevant?
  - Human data?
  - Dose escalation
- Maximum effective dose
- Route of administration
- Medical device needed for administration?

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Process- same degree of safety and effect every time

The same starting material should give the same product every time we manufacture, right?

<u>But</u>

- Usually different starting material
- Biological material will vary!
- Can you do exactly the same thing every time in the lab?

<u>So</u>

- How much variation can we accept without it affecting safety and effect?

How do we get a consistent process?

GMP: Rules and regulations for manufacturing of drugs

Good **M**anufacturing **P**ractice

Why GMP?

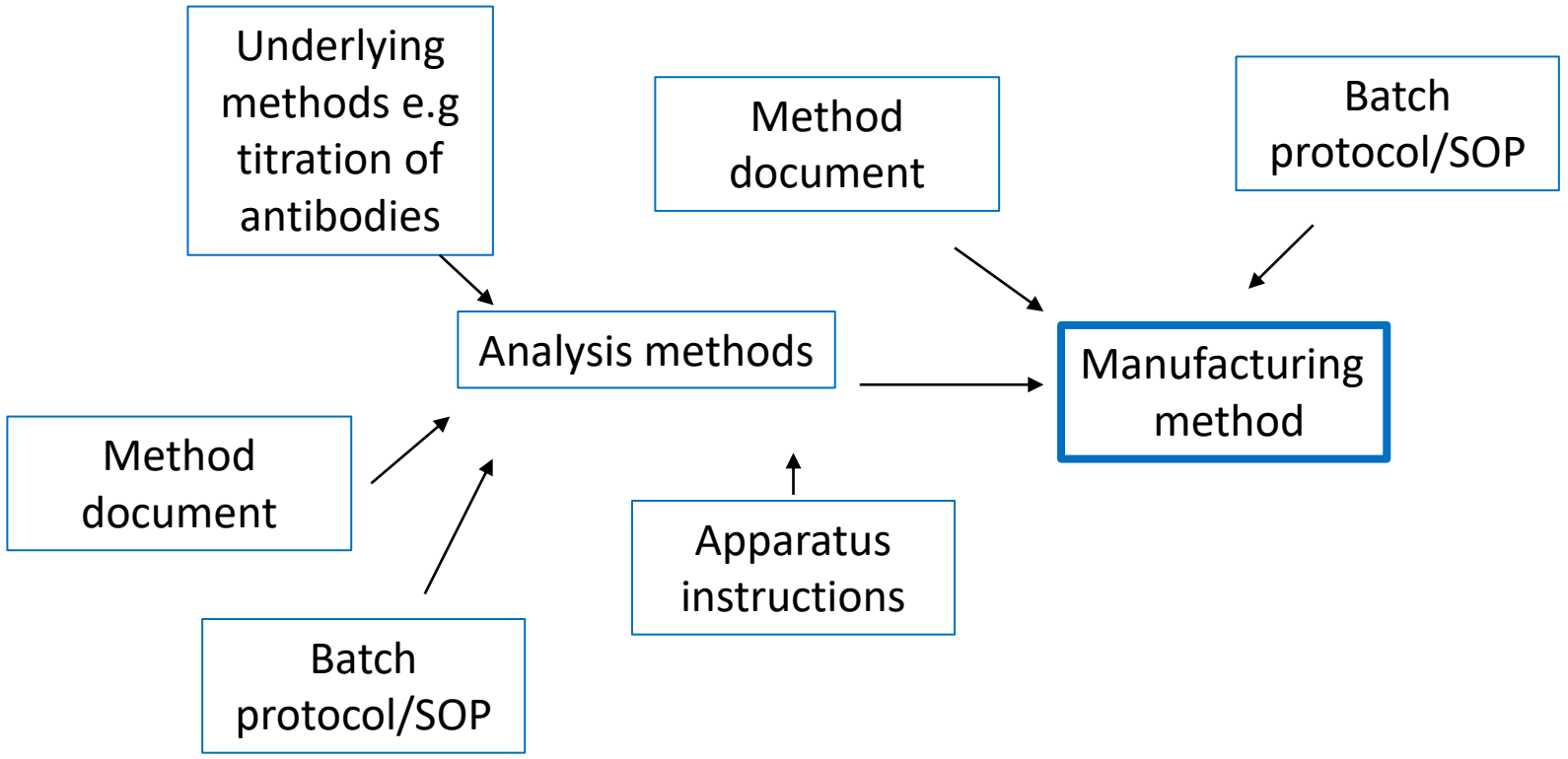
To eliminate the ”human factor”

- **Documentation of everything**
  - Production associated documents (≥70)
- **Strict protocols to follow- signature needed for each step**

To control for biologic variation

- **Controls at every step**

GMP documents for manufacturing



GMP documents for manufacturing- example

KAROLINSKA UNIVERSITY HOSPITAL

Karolinska Cell Therapy Center
Vecura

TM 173:1 Fetal mesenchymal stem cells
Drug Product
Batch # YYYYMMDD-173:2/CSn/DPn

Results reviewed by	Results approved by
Date *Prod. manager*	Date *QP*

Batch number DP	
Clinical trial	BOOSTB4 EudraCT 2015-003699-60
Batch number CS	
Cell processing performed by (name, signature)	

Production room	Cell room 5	**LAF inv#**	**9001018**
Room status verified (date, sign)	Cleaning OK	Room tests OK	Line clearance OK

CS #	Place label from CS vial here: Thawed Date…….. Time……	Label matches CS information above **Sign**

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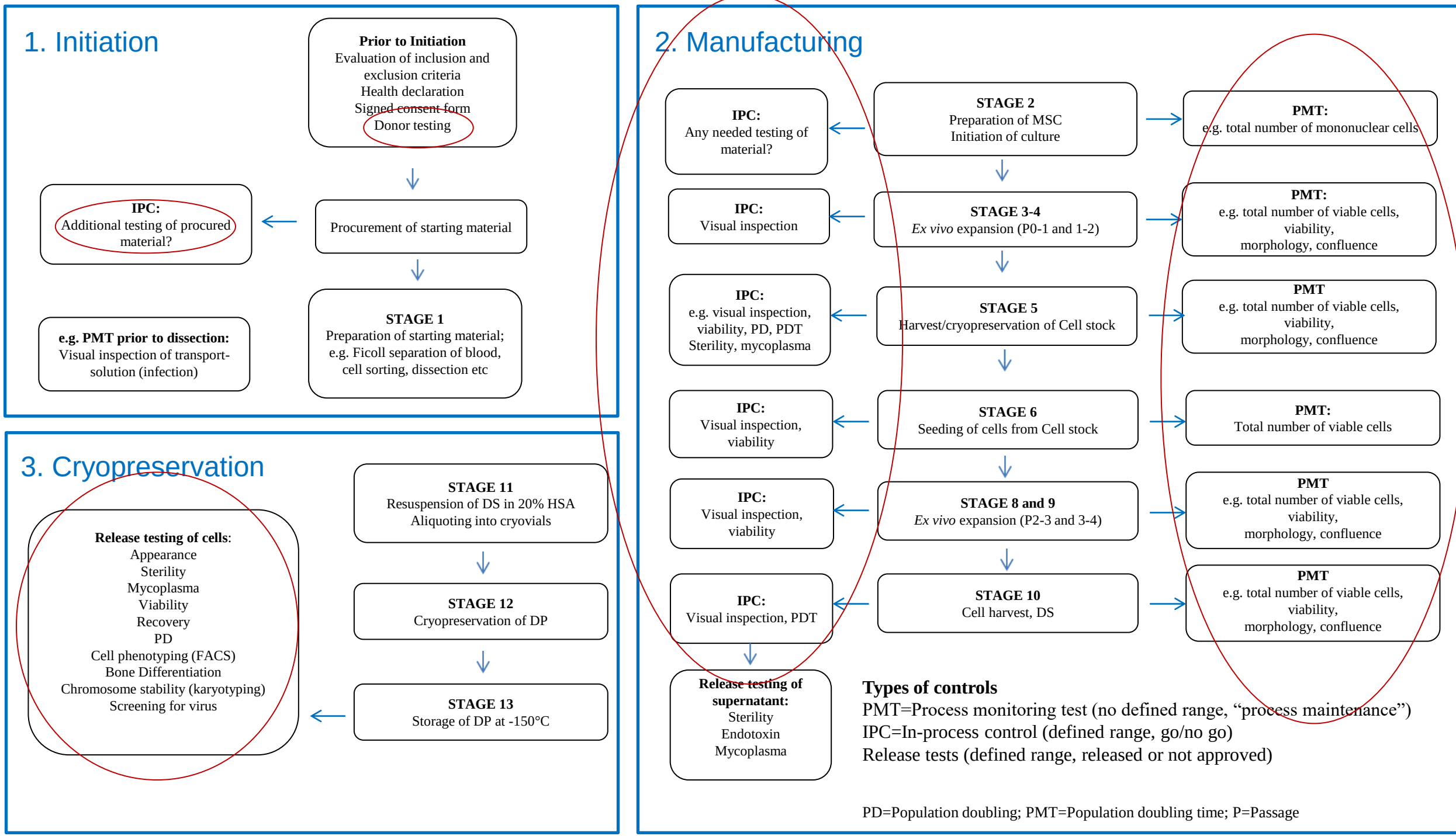
Vecura

TM 173:1 Fetal mesenchymal stem cells
Drug Product
Batch # YYYYMMDD-173:2/CSn/DPn

	Seeding of fMSC from Cell Stock (CS)	Date, Sign
1.	Day: 0 Time: …… One vial of cells from CS (label controlled on page 1) is thawed according to appendix 10a to TM 173 Fetal mesenchymal stem cells. Cells from one vial is normally enough to seed two 300 cm^2 flasks with 3000-5000 cells/cm^2	
2.	The cells are counted according to appendix 10a to M173. Total volume of cells (V2): _____ ml Dilution 10x: 50µl + 450µl 0,1% Eosin Y solution (M10, dil 1:5) **Viable:** Cell count per A square: _____ Mean cell number/A square: _____Conc. (C2a): _____ x 10^6 cells/ml **Total:** Cell count per A square:_____ Mean cell number/A square: _____Conc. (C2b): _____ x 10^6 cells/ml **Viability: _____%** Total number of viable cells (C2axV2): _____ x 10^6 cells	
3.	Time: …… Cells are seeded (P2) in culture flasks in CM according to Appendix 10a to M173. Target cell density: 3000 - 5000 cells/cm^2 Add …… ml to the cellsuspension Total volume…… ml Number of 300 cm^2 flasks (M2): _____ Seeding _____ ml cell suspension/flask Cell density: _____ cells/cm^2 Incubate in 37°C, 5 % CO_2 according to Appendix 10a	
4.	Day: …… Time: …… Aspirate off the medium and replace with an equal volume of fresh CM. Incubate the cells in 37°C, 5 % CO_2 according to Appendix 10a.	


1. Initiation
Prior to Initiation
Evaluation of inclusion and exclusion criteria
Health declaration
Signed consent form
Donor testing
Procurement of starting material
IPC:
Additional testing of procured material?
e.g. PMT prior to dissection:
Visual inspection of transport-solution (infection)
STAGE 1
Preparation of starting material;
e.g. Ficoll separation of blood, cell sorting, dissection etc
2. Manufacturing
STAGE 2
Preparation of MSC
Initiation of culture
IPC:
Any needed testing of material?
PMT:
e.g. total number of mononuclear cells
STAGE 3-4
Ex vivo expansion (P0-1 and 1-2)
IPC:
Visual inspection
PMT:
e.g. total number of viable cells, viability, morphology, confluence
STAGE 5
Harvest/cryopreservation of Cell stock
IPC:
e.g. visual inspection, viability, PD, PDT
Sterility, mycoplasma
PMT
e.g. total number of viable cells, viability, morphology, confluence
STAGE 6
Seeding of cells from Cell stock
IPC:
Visual inspection, viability
PMT:
Total number of viable cells
STAGE 8 and 9
Ex vivo expansion (P2-3 and 3-4)
IPC:
Visual inspection, viability
PMT
e.g. total number of viable cells, viability, morphology, confluence
STAGE 10
Cell harvest, DS
IPC:
Visual inspection, PDT
PMT
e.g. total number of viable cells, viability, morphology, confluence
Release testing of supernatant:
Sterility
Endotoxin
Mycoplasma
Types of controls
PMT=Process monitoring test (no defined range, “process maintenance”)
IPC=In-process control (defined range, go/no go)
Release tests (defined range, released or not approved)
PD=Population doubling; PMT=Population doubling time; P=Passage
3. Cryopreservation
STAGE 11
Resuspension of DS in 20% HSA
Aliquoting into cryovials
STAGE 12
Cryopreservation of DP
STAGE 13
Storage of DP at -150°C
Release testing of cells:
Appearance
Sterility
Mycoplasma
Viability
Recovery
PD
Cell phenotyping (FACS)
Bone Differentiation
Chromosome stability (karyotyping)
Screening for virus

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Product- same product every time

Process must be repeatable

In our toolbox we have:

- Selected **analysis methods**

- The **product specification**- states the accepted range of a desired component in the end product

- **Validation**- in order to show that every time we manufacture, we get the “same” product

The Product Specification

- The product specification states what demands you have on the product (what you analyze the product for)
  - ➢ e.g. sterile, amount of viable cells, type of cell, potency etc
  - ➢ acceptance ranges for the release controls
- Must be fulfilled every time we manufacture

Specifications for the Control of Product xx

Test	Method	Acceptance criteria	Comments
Population doublings	Cell count	≤ 13 cell doublings from passage 1	Performed on DP after thawing
Viability (at thawing)	Cell count	≥ 70%	
Recovery (at thawing)	Cell count	≥ $3.5x10^6$ viable cells/mL	
% viable MSC % contaminating cells	Flow cytometry analysis	≥ 85% for CD73& CD90 ≤ 5% for CD31 ≤ 5% for CD45 ≤ 5% for HLA class II	
Bone differentiation	Bone differentiation assay (*in vitro*)	≥ 2.5-fold differentiation over negative control	
Chromosome stability	Karyotyping	46,XX or 46,XY	
Virus screening	PCR	Negative for virus x, y	
Sterility	Ph.Eur. 2.6.1	Sterile	Performed on DP[4] after thawing (cells) and on DS before freezing (supernatant)
Mycoplasma	Ph.Eur. 2.6.14	No mycoplasma detected	
Endotoxin	Ph.Eur. 2.6.7 (qPCR)	≤0.5EU/mL	On DS before freezing (supernatant)
Appearance	Visual inspection	Colourless cell suspension, free of visible particulate matter	On DP before freezing (cell suspension)

Validation

So we know from the specification what we need to achieve, let´s just run the samples then…..

BUT

How do you know that a given test result is ”true”?

- Validation of main process and of all analysis methods used in your process

Validation- an example

Phenotyping of cells with Flow Cytometry

CD90+ cells
Day 1 (%) 72, 69, 81
Day 2 (%) 73, 79, 75

-repeated analysis of sample on same day

-repeated analysis of sample on different days

-include cells with ”known” expression of markers **at all sharp runs**

Precision of your process is measured by % CV

Coefficient of **v**ariation or relative standard deviation
-mean value of your measurements divided by the standard deviation gives the %CV

Mean=74,8
SD= 4,1
%CV=18,2

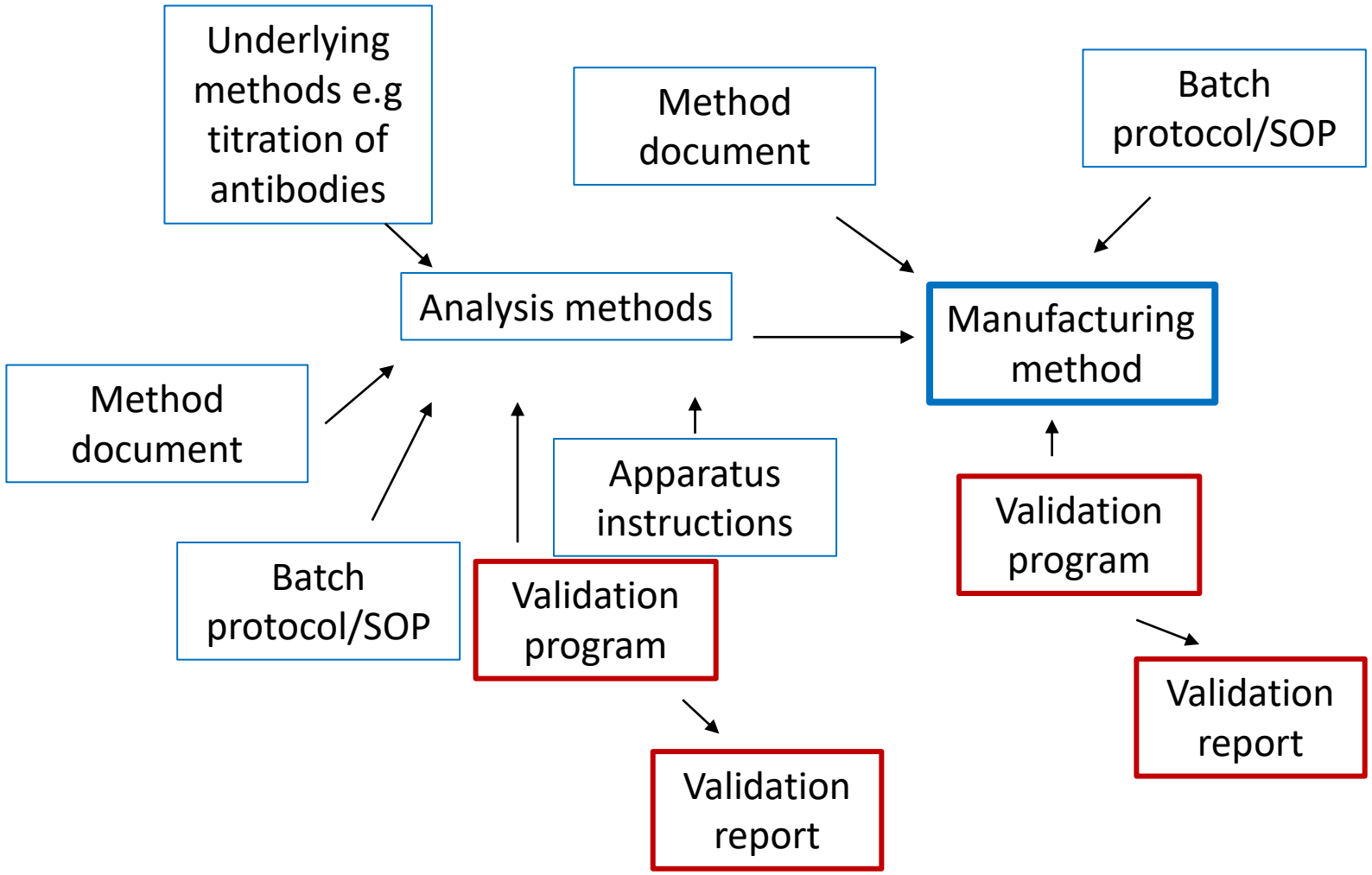
-demonstrate ”linearity” when diluting the cells

-sensitivity of method, how few cells expressing the relevant marker can you detect

-same results with different operators

- same results with different instruments

Validation of manufacturing



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Stability- for how long and how can the product be stored

- Stability of starting material
- Long term stability of drug product- shelf life
- Need for reconstitution step?
- In-use stability
- Stability during transport
- Shelf life extension plan

Example stability program

Table 2.1.P-22. Outline of stability study

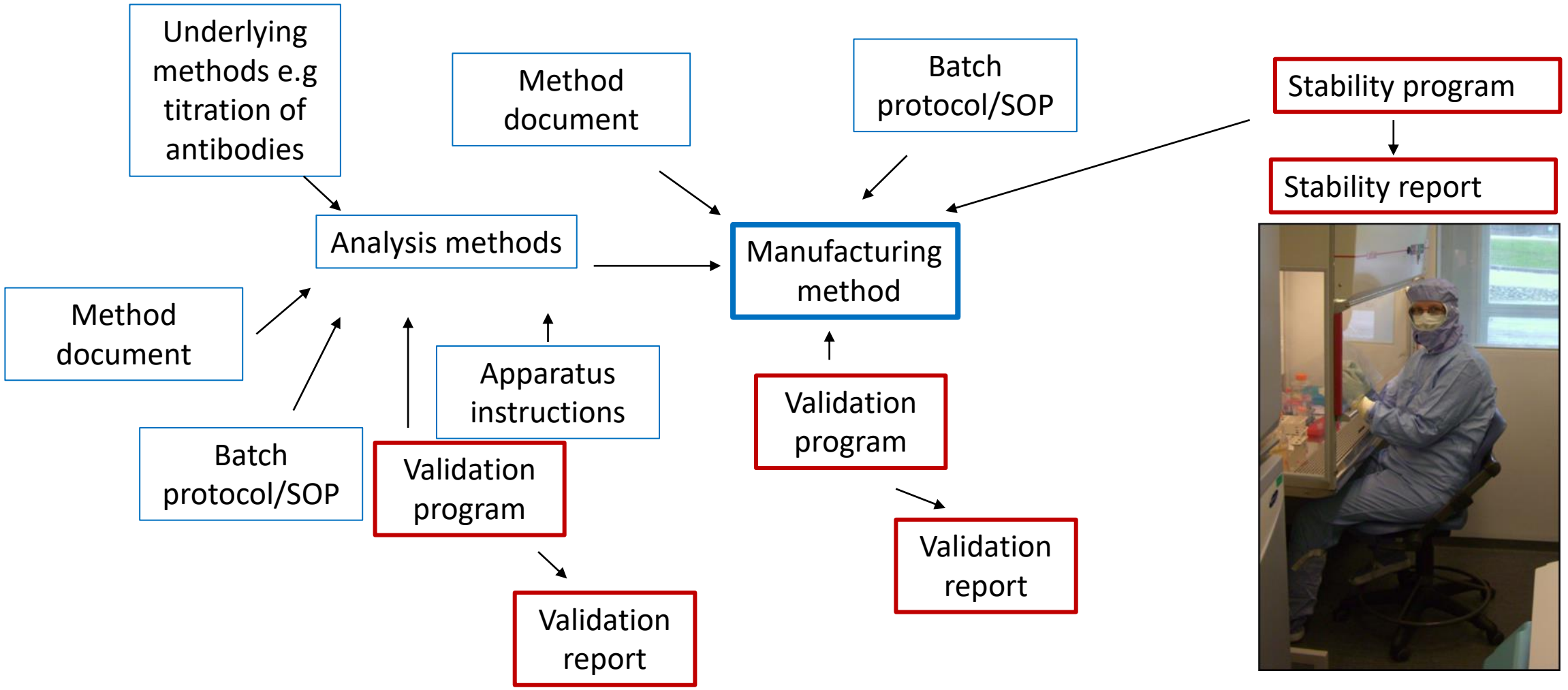
Time points (months)	0-1[1]	3	6	9	12	18	24	30	36
Package integrity (pass/fail) [2]	x	x	x	x	x	x	x	x	x
Sterility (sterile/not sterile)	x		x		x		x		x
Viability (≥ 70%)	x	x	x	x	x	x	x	x	x
CD73/CD90 cells (≥ 85%)	x		x		x		x		x
CD31/CD45/HLA II (≤ 5%/marker)	x		x		x		x		x
Bone differentiation (≥ 2.5-fold differentiation over negative control	x	x	x	x	x	x	x	x	x

[1]Data from this time point is considered the start value (release data) [2]Includes cryo vial integrity; appearance, cap closed properly and cap tightly closed
Dark cell=not performed at this time point

Table 2.1.P-24. Proposed shelf-life extension plan

Available development batch stability data for real time (months)	Available clinical batch stability data for real time (months)	Proposed shelf life (months)
6	0	12
9	0	12
9	6	12
12	9	18
18	12	24
24	18	30
30	24	36
36	30	36
	36	36

Stability of manufactured product



Now we are on top of manufacturing

- ✓ A validated and reproducible manufacturing method
- ✓ A product that fulfills the set specification
- ✓ An ongoing stability program and a shelf life extension plan

And by the way…….
Risk assessments
Starting material
Raw material
GMP grade reagents
Serum
etc

And onwards to the clinical trial application……

What is needed to go from test tube to candidate drug

- Clinical trial application
  - Investigators medicinal product dossier (IMPD)
  - Investigator´s Broschure (IB)
  - Clinical Trial Protocol (CTP)
- Adaptation to Good manufacturing practice (GMP)

  → Production associated documents (≥70)
- Ethics application
  - Donor information and agreements
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Guidelines to regulate what has to be done :
https://www.ema.europa.eu/en/human-medicines-regulatory-information

IMPD- Investigational Medicinal Product Dossier

- Includes summaries of information related to the quality, manufacture and control of the drug, aka data from your validation

Challenges :

- Handling variations in production
- Drug substance vs Drug product
- Long term risks e.g. tumor formation

Useful link:

- https://atmpsweden.se/atmp-regulatory-guide/document-templates/

IB- Investigator´s Broshure

- Purpose is to compile data relevant to studies in human subjects gathered during preclinical and other clinical trials

- Introduce key aspects and safety measures such as dose, frequency of dosing interval, methods of administration, safety monitoring procedures

Challenges:

- Usually no toxicity data, pharmacokinetic data, and pharmacodynamic
- Dosing and cell types not always relevant in animal models

➢ Biodistribution

Useful link:

- https://atmpsweden.se/atmp-regulatory-guide/document-templates/

CTP- Clinical Trial Protocol

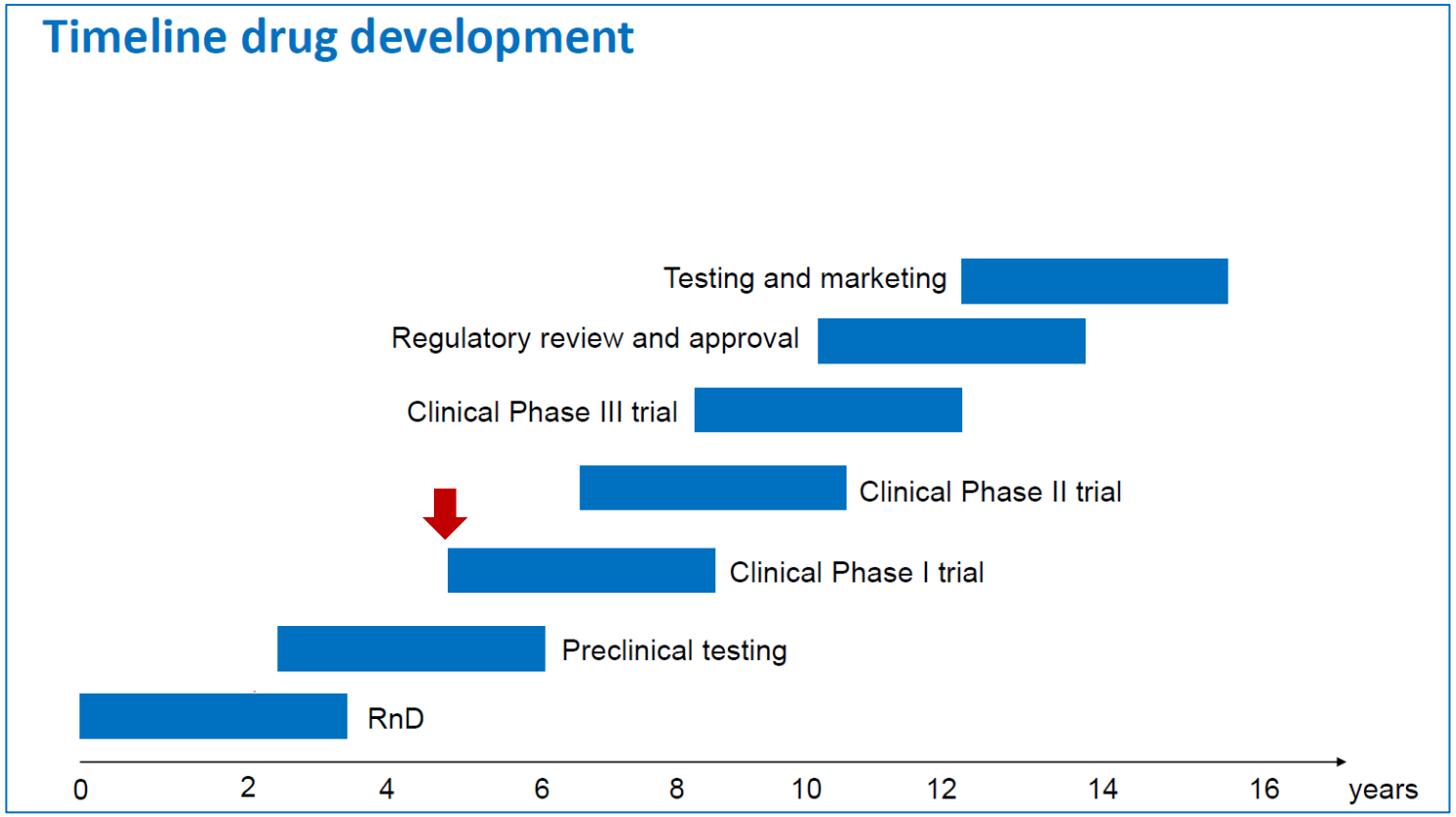
- Description of the planned clinical study in detail; how it will be conducted
  - Study design, methodology, statistical considerations, sampling
- Ensures the safety of the trial subjects and integrity of the data collected

Challenges :

- Risk benefit analysis

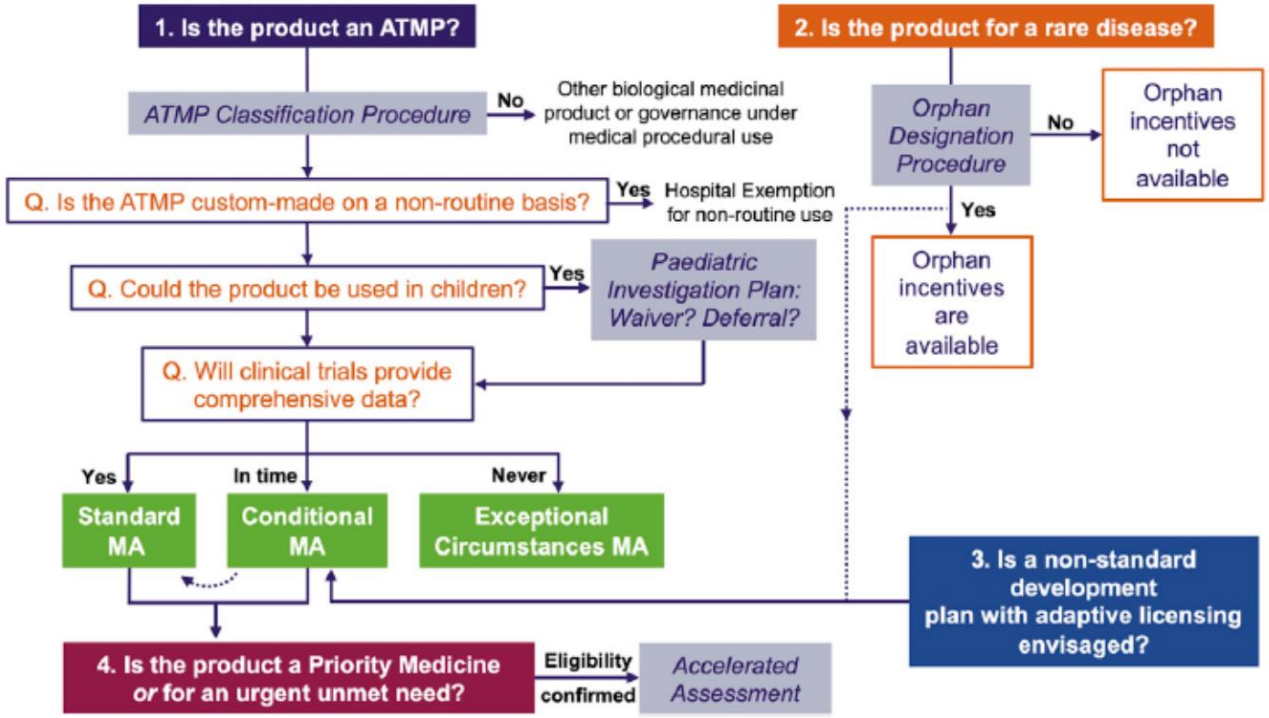
Useful link:
https://atmpsweden.se/atmp-regulatory-guide/document-templates/

You can do it!


Timeline drug development
Testing and marketing
Regulatory review and approval
Clinical Phase III trial
Clinical Phase II trial
Clinical Phase I trial
Preclinical testing
RnD
0
2
4
6
8
10
12
14
16
years

Next steps to consider:

- Regulatory strategy
  - ODD (Orphan drug designation)
  - Prime (Priority medicine)
  - PIP (Pediatric investigation plan)
- CMC strategy
- Commercialize or stay within academy
  - IP
  - Exit or ”do it yourself”?
  - Financing
  - Reimbursement strategy

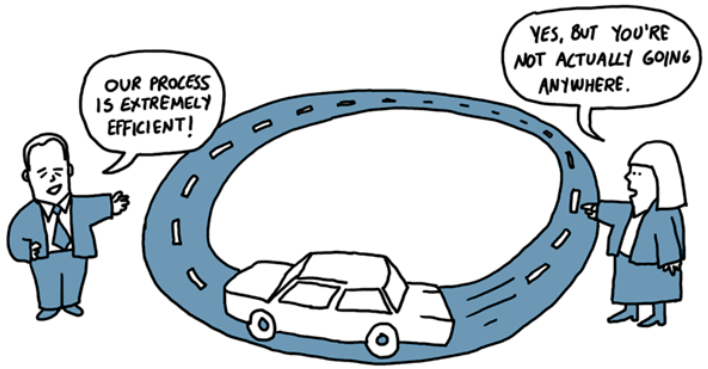


From Detela et al. Mol Ther Methods Clin Dev 2019

ATMP golden rule: The process is the product

CMC strategy:

- Make changes early during the development
  - → Scale up-how much can be handled without compromising the quality of the product
  - → Supply chain
- Every change needs justification
- Scientific advice



What is Scientific advice

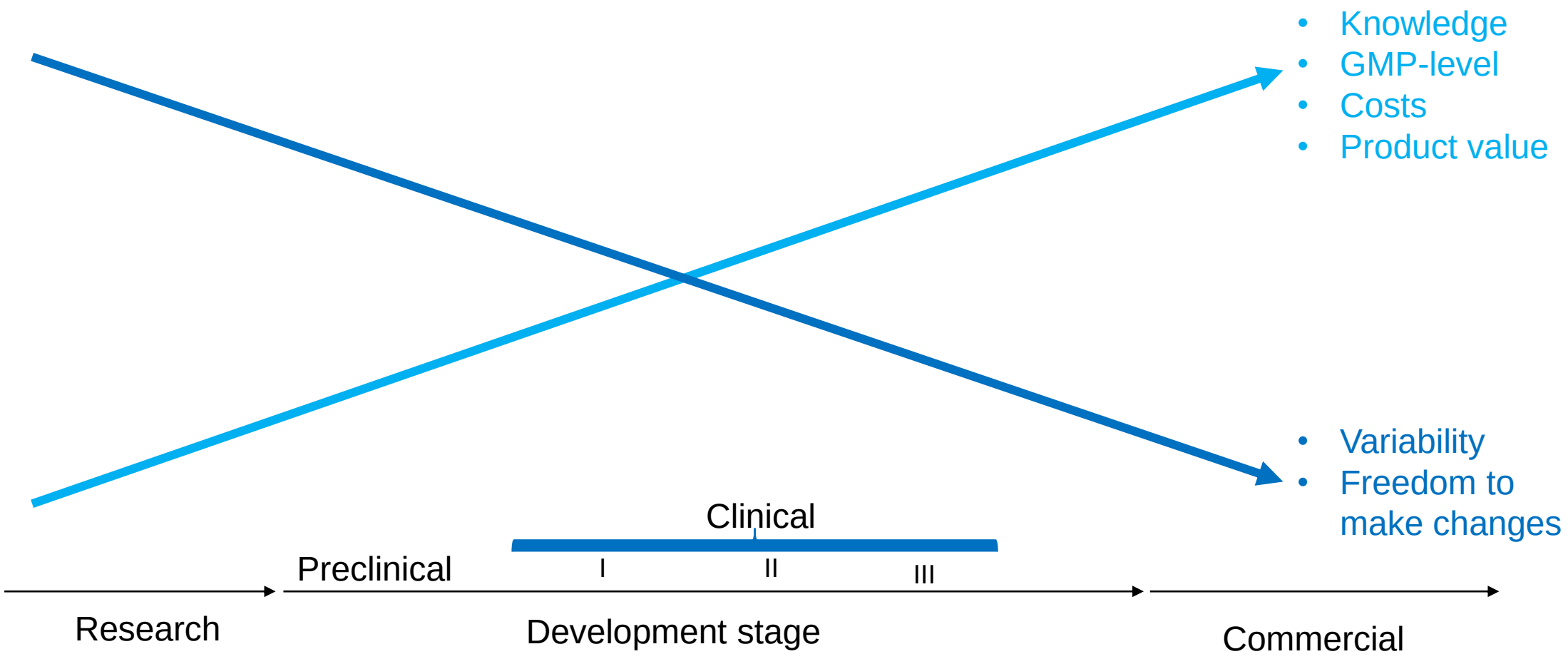
Possibility to discuss your questions with the regulators

- Check timeline, costs and demands
- What need to be prepared?
  - Briefing book with the ”questions” together with an ”answer”, stating the investigators (your) position on the subject
- Authorithies do not tell you what to do and how to do it!

  N.B. You get the answers you ask for!

- ✓ Still worth the effort and the money

Basic drug development- summary



Thank you!