Formulation considerations for T-cell therapies

Chris van der Walle, PhD
Director, Formulation
Cell & Gene Therapy

Academy of Pharmaceutical Sciences
University of Strathclyde
Cell and gene therapy: from concept to clinical use
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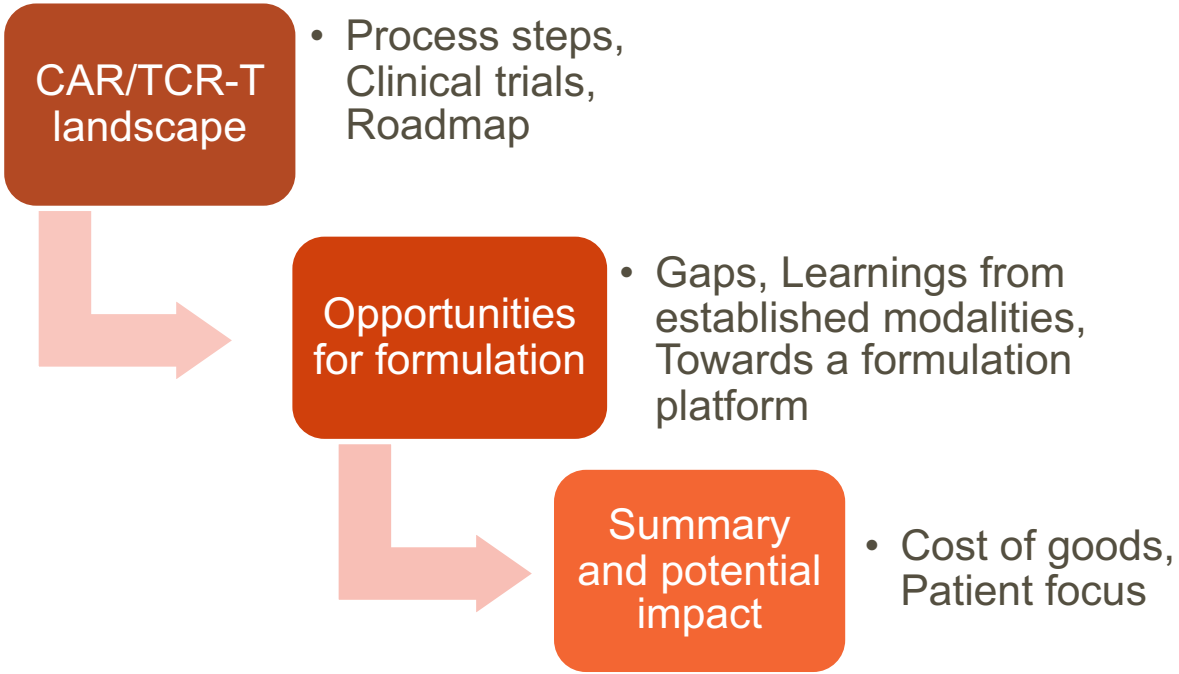
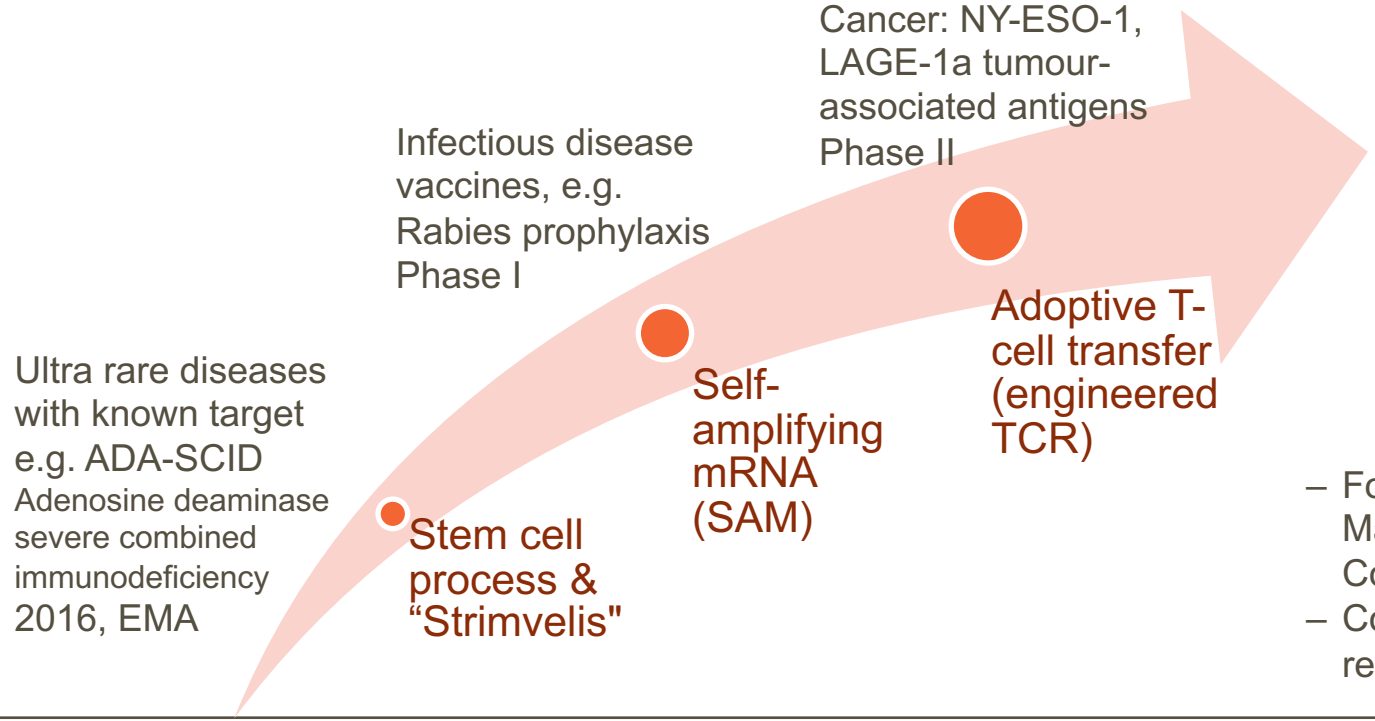
Outline


CAR/TCR-T landscape
• Process steps, Clinical trials, Roadmap
Opportunities for formulation
• Gaps, Learnings from established modalities, Towards a formulation platform
Summary and potential impact
• Cost of goods, Patient focus

Delivering Innovative, Break-Through Gene Therapies

Unmet needs in several therapeutic areas



- Focus on Chemistry, Manufacturing, and Controls (CMC)
- Cost of Goods (COGs) remains challenging

Early successes in T-cell immunotherapies

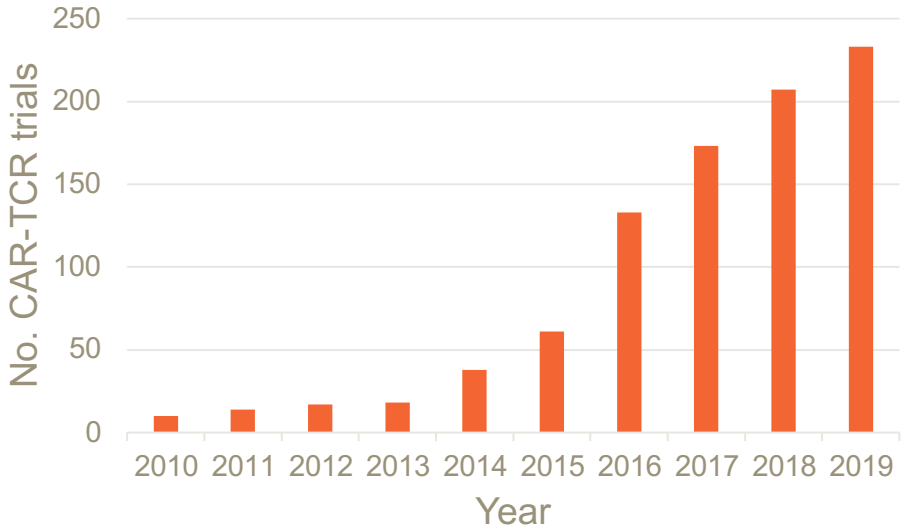
FDA-approved products to date are autologous CAR-Ts

- Chimeric antigen receptor therapy, CAR-T: recognise tumour cell surface antigens
- All target CD19 to treat B-cell malignancies, *except* Abecma® which targets B-cell maturation antigen for multiple myeloma
- All presented as suspension in one or more bag(s) for infusion, *except* Breyanzi which consists of two components (1:1 CD4:CD8 cells), each supplied separately in 1-4 single-dose 5 mL plastic vials
- Adult dose is typically from 0.5 to 6.0 × 10^8 CAR T cells, unless by weight
  - Kymriah® (tisagenlecleucel), Novartis, 2017
  - Yescarta® (axicabtagene ciloleucel), Kite/Gilead, 2017, and-
  - Tecartus™ (brexucabtagene autoleucel), Kite/Gilead, 2020
    - both dosed with 2 × 10^6 CAR T cells per kg of body weight
  - Breyanzi® (lisocabtagene maraleucel), Juno/BMS, 2021
  - Abecma® (idecabtagene vicleucel), Bluebird/BMS, 2021

CAR/TCR is prevalent among all cell therapy modalities

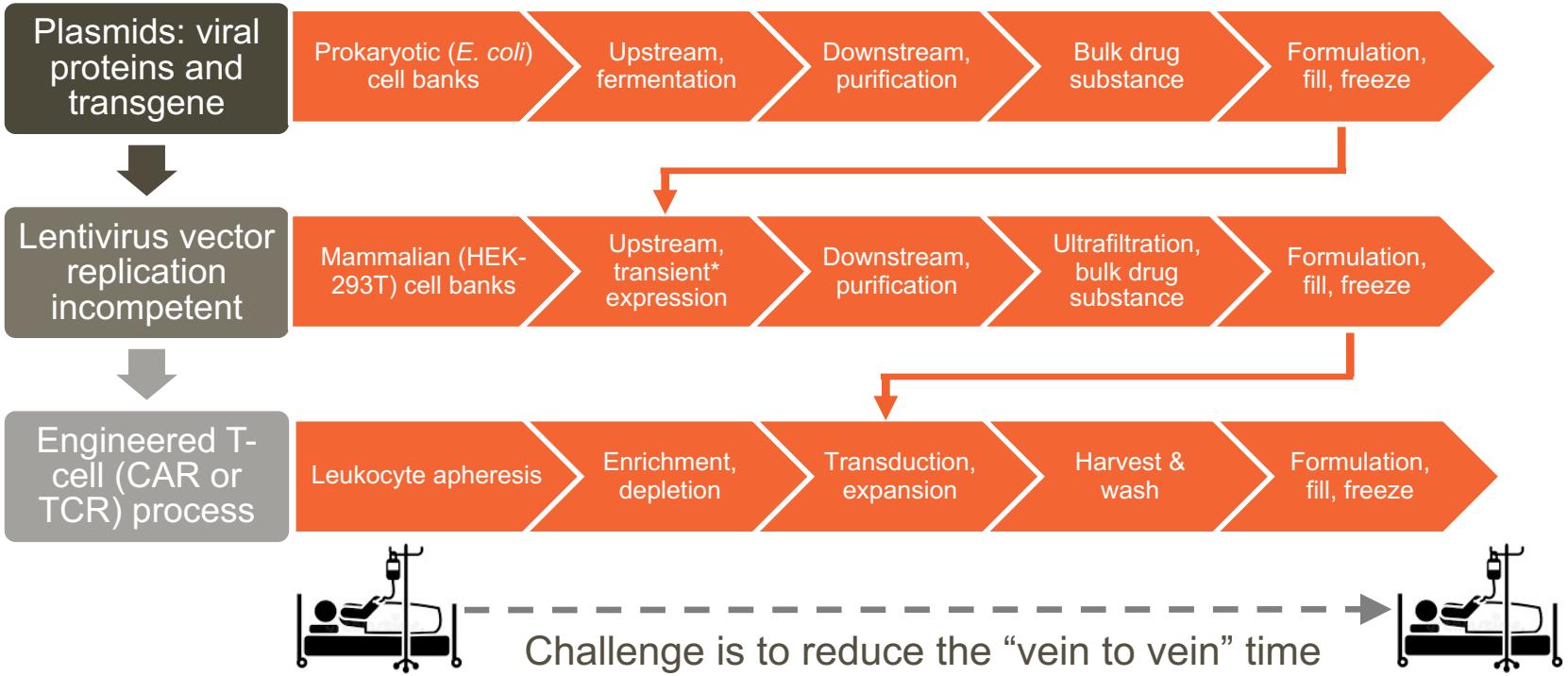
Mostly **autologous** T-cell therapies

- Early years were predominantly academic-led studies
- Last few years has seen strong interest from biotech and large pharma companies
  - especially since success of anti-CD19 CAR-T
  - ~50% of trials are academic-company collaborations
- Expect the proportion of **allogenic** therapies to increase



Establishing a platform process for autologous T-cells

Oncology



Challenges for T-cell design, engineering

Formulation strategy must align to future design criteria

- Standardization of apheresis material presents a complex challenge
  - platform process is built on apheresis leukocytes from healthy donors, distinct from patients
  - inherent variability: phenotypes, transduction efficiencies, expansion, exhaustion
  - immunogenicity associated with CAR/TCR construct

- Solid tumours are more challenging than hematological cancers for T-cells:
  - must find, enter and survive in the tumour
    - but poor blood flow, hypoxic and immunosuppressive environment
  - solutions include
    - dual CARs, address antigen heterogeneity & downregulation
    - checkpoint inhibitors, delay the onset of T-cell exhaustion

Formulation

of the T-cell drug product

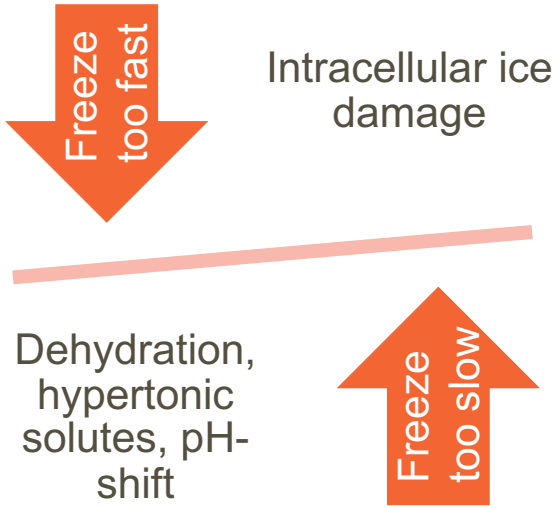
Formulations used in marketed CAR-T products

	Kymriah (DLBCL, adults)	Yescarta
Dose (CAR+ T-cells)	1-3 bags for up to 6 x 10^8	1 bag, ~68 ml, 0.4 to 2 x 10^8
Shelf-life, frozen and in-use	9 months, -120°C 0.5 hours, room temp	12 months, -150°C 3 hours, room temp
Formulation vehicle	1:1 Cryostor10 : human serum albumin (HSA), 0.9% NaCl (5% dimethyl sulphoxide, DMSO)	Glucose, NaCl, HSA, DMSO dextran- 40, Na.gluconate, Na.acetate, Na.caprylate

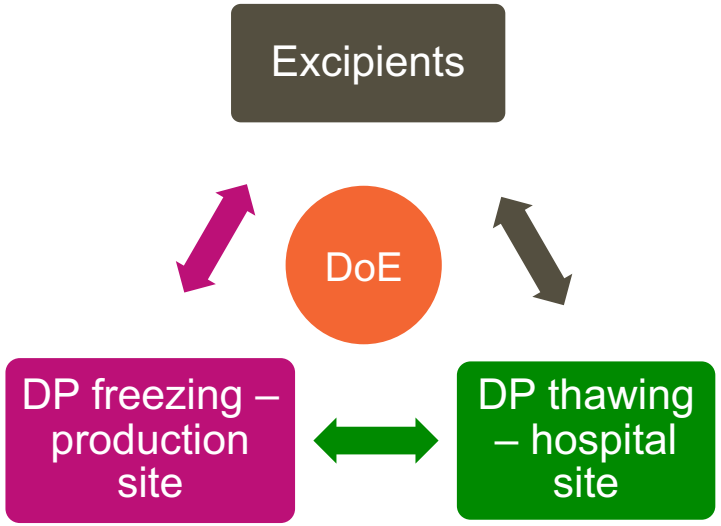
- Primary packaging: ethylene vinyl acetate (EVA) infusion bag with polyvinyl chloride (PVC) tubing. Secondary packaging: aluminium cassette.
- Administration: thaw next to patient bedside?
- COGs: proprietary excipient, components?

Control of freeze/thaw process for autologous T-cells

Formulation must include cryoprotectant in final wash step of cell process



– Consider cell metabolism and lipid phase transition in cell membrane

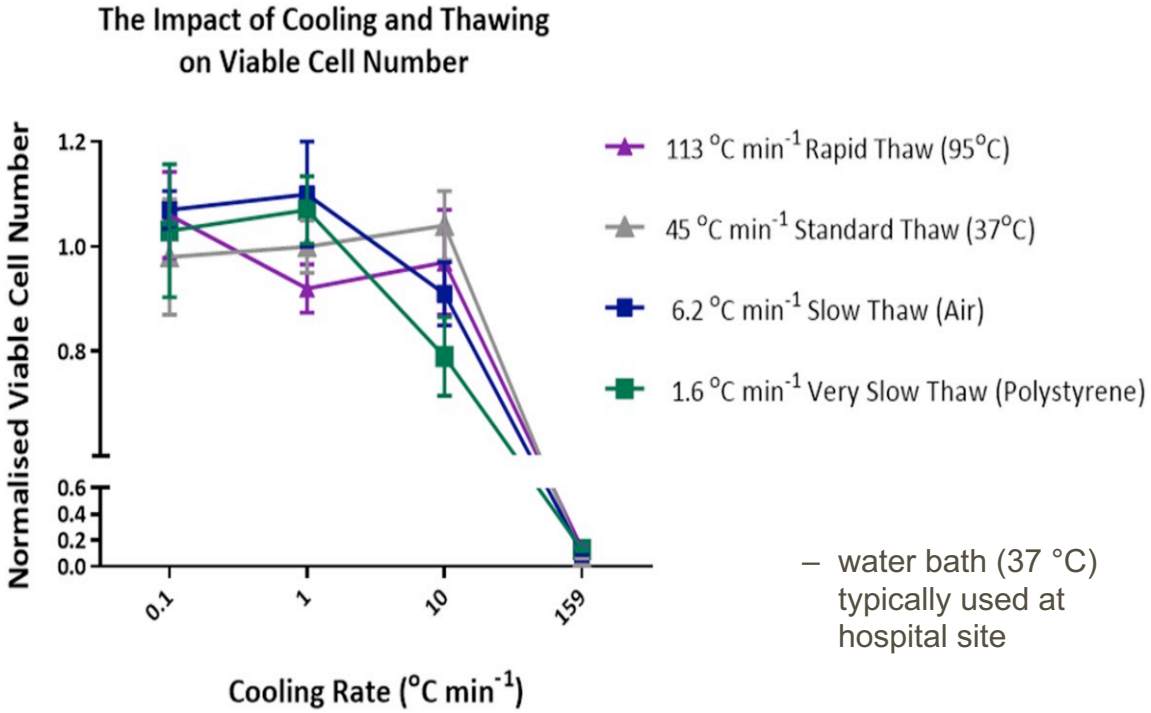


– Design of Experiments (DoE) to assess formulation robustness of T-cell drug product (DP)

Impact of different cool / thaw rates on T-cell viability

Baboo, *et al.*, Scientific Reports, 2019, 9:3417

- Thaw rate has little impact if cool rate ≤ −1 °C/min
- most cell damage for slow thaw following rapid cool, ≥ -10 °C/min
- correlated with ice recrystallisation during slow warm - mechanical disruption?
- DMSO used as cryoprotectant



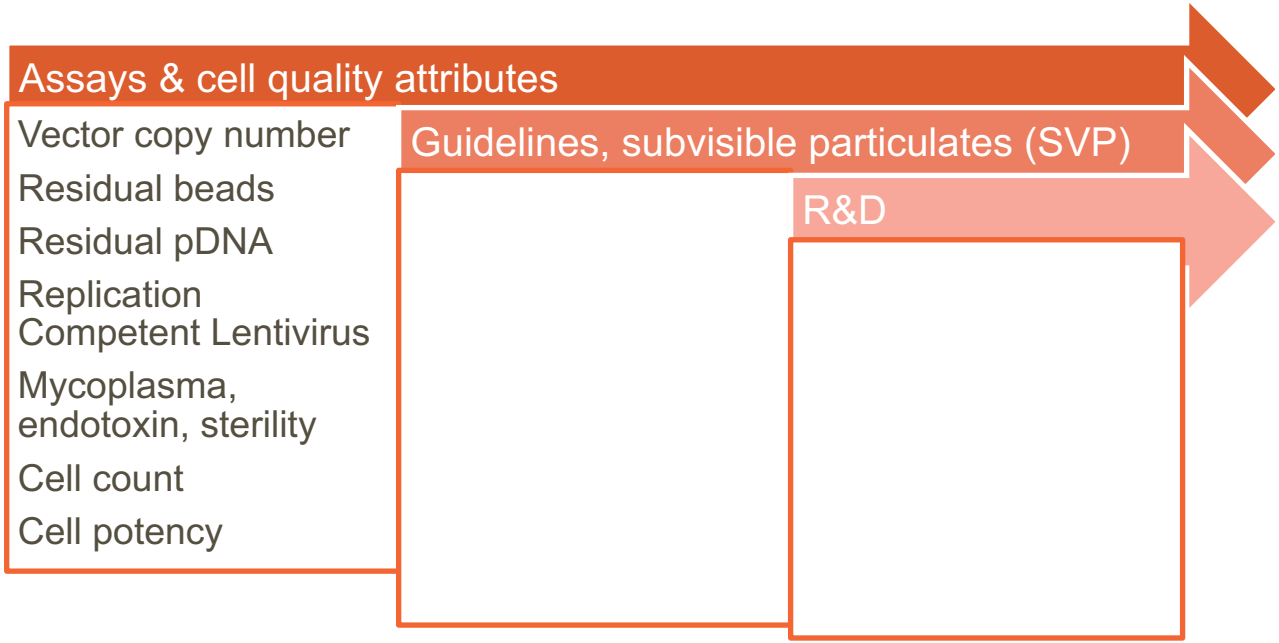
- water bath (37 °C) typically used at hospital site

Are particulate assays useful in T-cell formulation?

Design of stability indicating tests for T-cell DP

Challenges

- Limited material, time points are carefully chosen
- No accelerated/stress temps. or Arrhenius modelling (cf. ICH Q1 and Q5C-biologics)
- Cell assays are time consuming and technically difficult



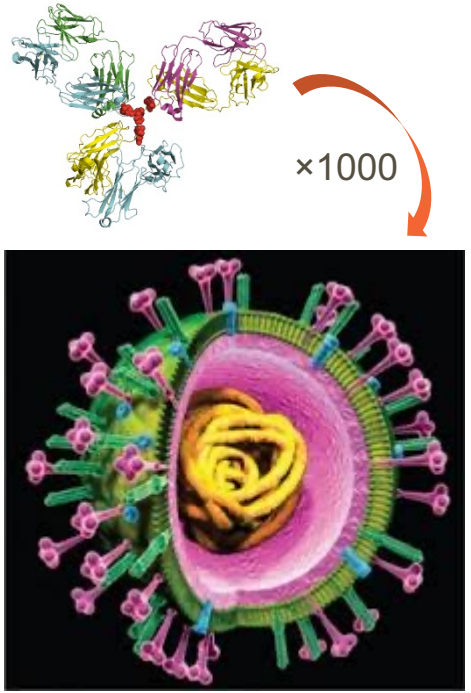
Formulation

of the lentivirus vector

The formulation of lentivirus vector is in its infancy

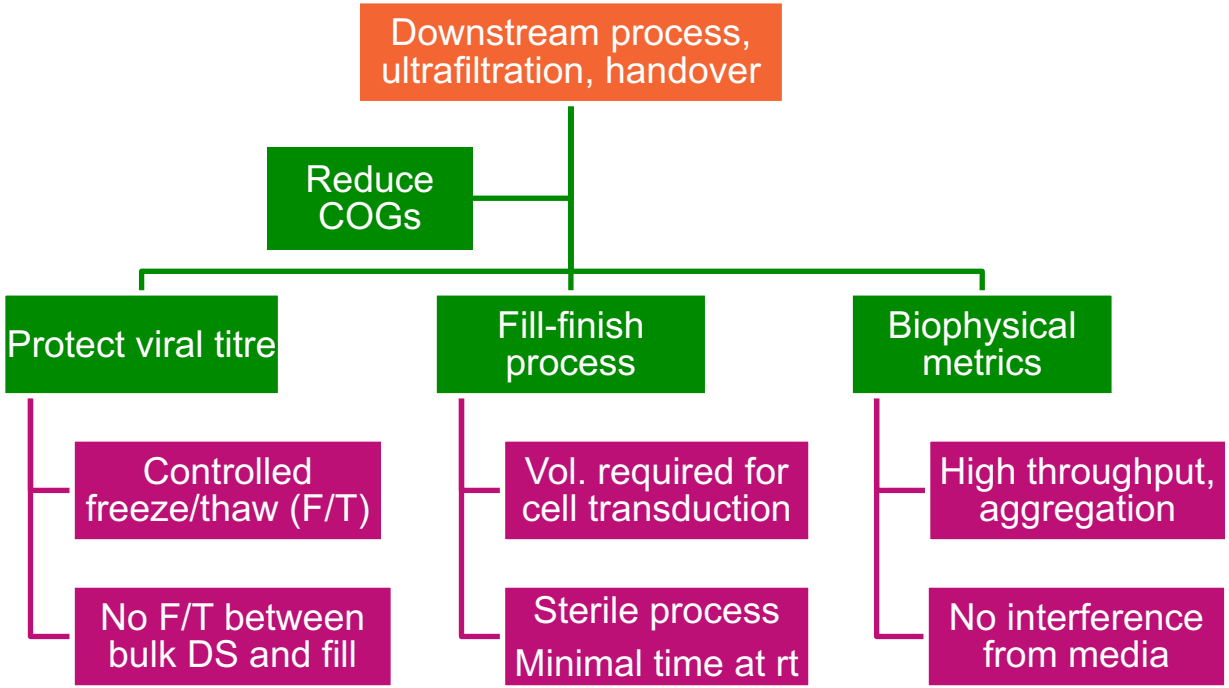
Compared to monoclonal antibody platforms

Monoclonal antibody	Lentivirus vector
150 kDa, 10 nm diam.	250 x 10^3 kDa, 80-120 nm diam.
Generally, pI ~8	Phospholipid membrane net negative, gp120 of VSV-G envelope net positive
Shear sensitive, 3 year shelf-life, 4-8 °C	Sensitive to freeze/thaw, high [NaCl]; store -80 °C
Formulate in histidine + sucrose, pH 6	Formulation compatible with cell process; proprietary media? lyophile?
Fill finish into vials, pre-filled syringes, etc.	Fill-finish: vial *vs* cryogenic bags



Gaps to be addressed in formulating lentivirus vector

Must achieve minimum criteria for ex-vivo transduction efficiency of T-cells



Fill-finish strategy for Lentivirus vector

Selection of primary container, transduction step compatible with cell process

Fill into vial
– manual inject

Manual/semi-automated cell process
- wave bag
- G-Rex

Automated cell process
- Quantum
- Prodigy

Fill into bag
– weld tubing to system
– maintain sterility

What plastic?

– Polyvinyl chloride (PVC), used for tubing
  – stiff but low temp. sensitive
– Plasticized PVC used for whole blood
  – 30% di(2-ethylhexyl)phthalate
    – leaches, acidifies, safety?
– Ethylene-vinyl acetate, EVA, selected
  – tough but flexible, puncture-resistant
  – cannot sterilise by heat

Scoping a suitable buffers for lentivirus vector

Strategy to avoid proprietary media and facilitate biophysical characterisation

Example Quality Attributes

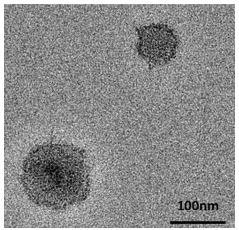
- Infectious *vs* physical titre
- Impurities: raw materials and process
- Adventitious agents
- Sterility, mycoplasma, endotoxin
- Appearance, pH, osmolarity

Virus-specific assays

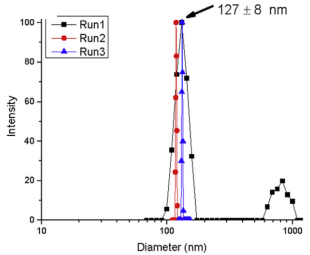
- Cell based e.g. infectivity
- ELISA, e.g. quantification p24 antigen

Formulation screen

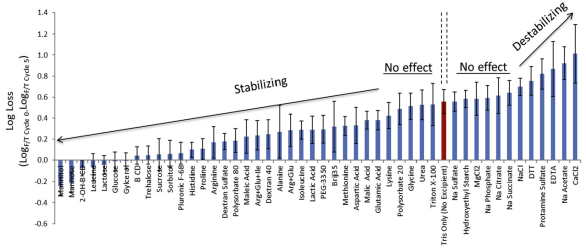
- New analytical methods measuring physical stability: size & number



Microscopy



Light scattering



High throughput, benchtop tech.

Kumru et al., J Pharm Sci., 2018, 107, 2764-74

Summary

Formulation strategy must align to future needs

- Lower COGs
  - labour costs est. 71% of manufacturing costs (Cell Gene Therapy Insights, 2018, 4, 1105-16)
  - materials costs 18%, most from apheresis, disposables, and virus
  - allogenic T-cells will decrease COGs
- Decrease vein-to-vein time
  - centralised *vs* decentralised (bedside) production
  - shorter end-to-end process times
  - cell transduction with non-viral systems
- The formulator has a role to play:
  - selection of excipients and automation
  - platform formulation fit to process
  - increase stability of cell and vector DP

Acknowledgements

Cell & Gene Therapy, GSK