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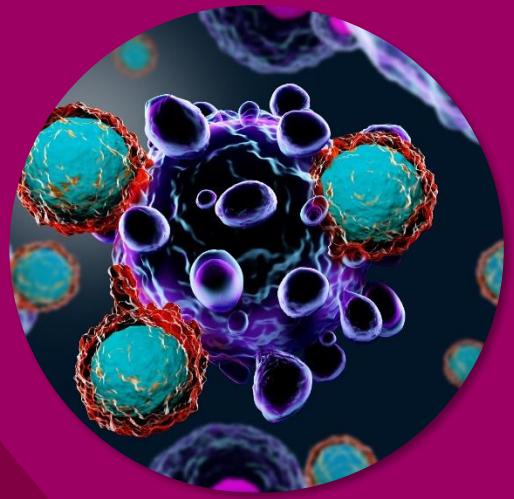
Challenge 32: Transgene Track

Sponsors
GSK and Novartis

Duration
Phase 1: six months, Phase 2: Up to three years

Budget

Phase 1: £100k; Phase 2: £1M



NC 3Rs National Centre for the Replacement Refinement & Reduction of Animals in Research

Challenge 32 - Transgene Track

Development of a sensitive, absolute quantification method for tracking AAV gene therapies and/or CAR-T cells *in vivo*

Launch Meeting

11 September 2019

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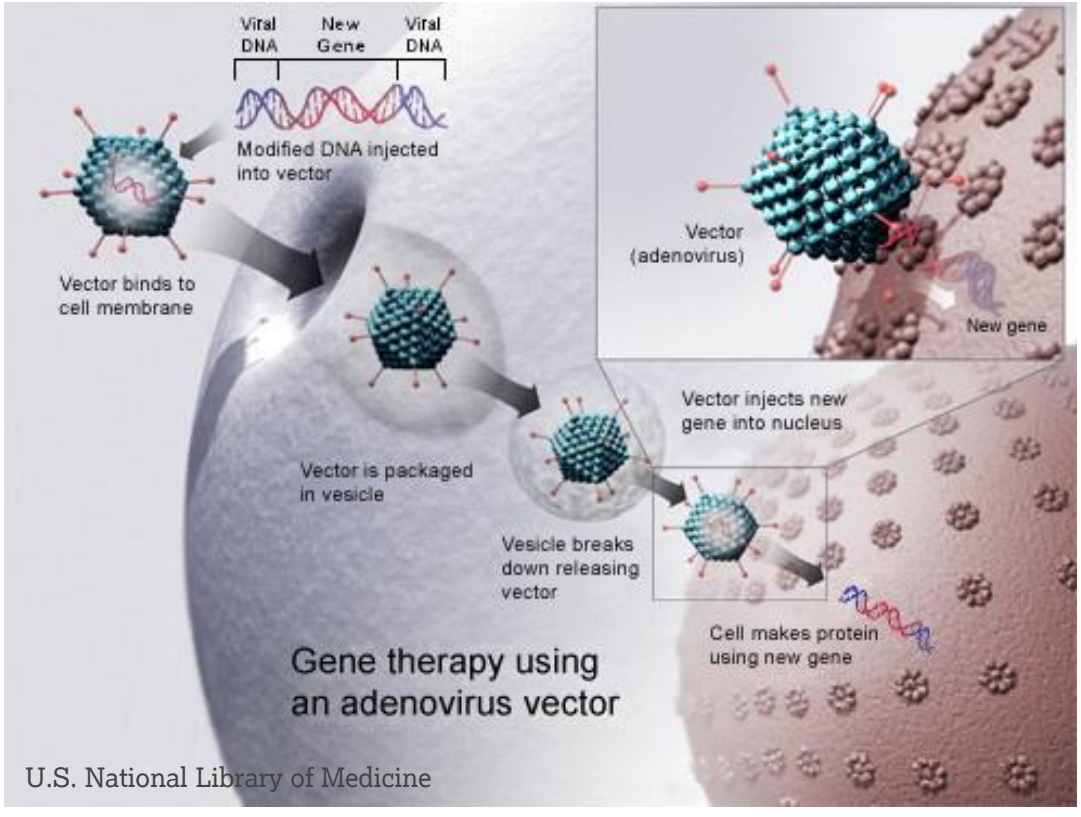
Mode of Action of Gene Therapies



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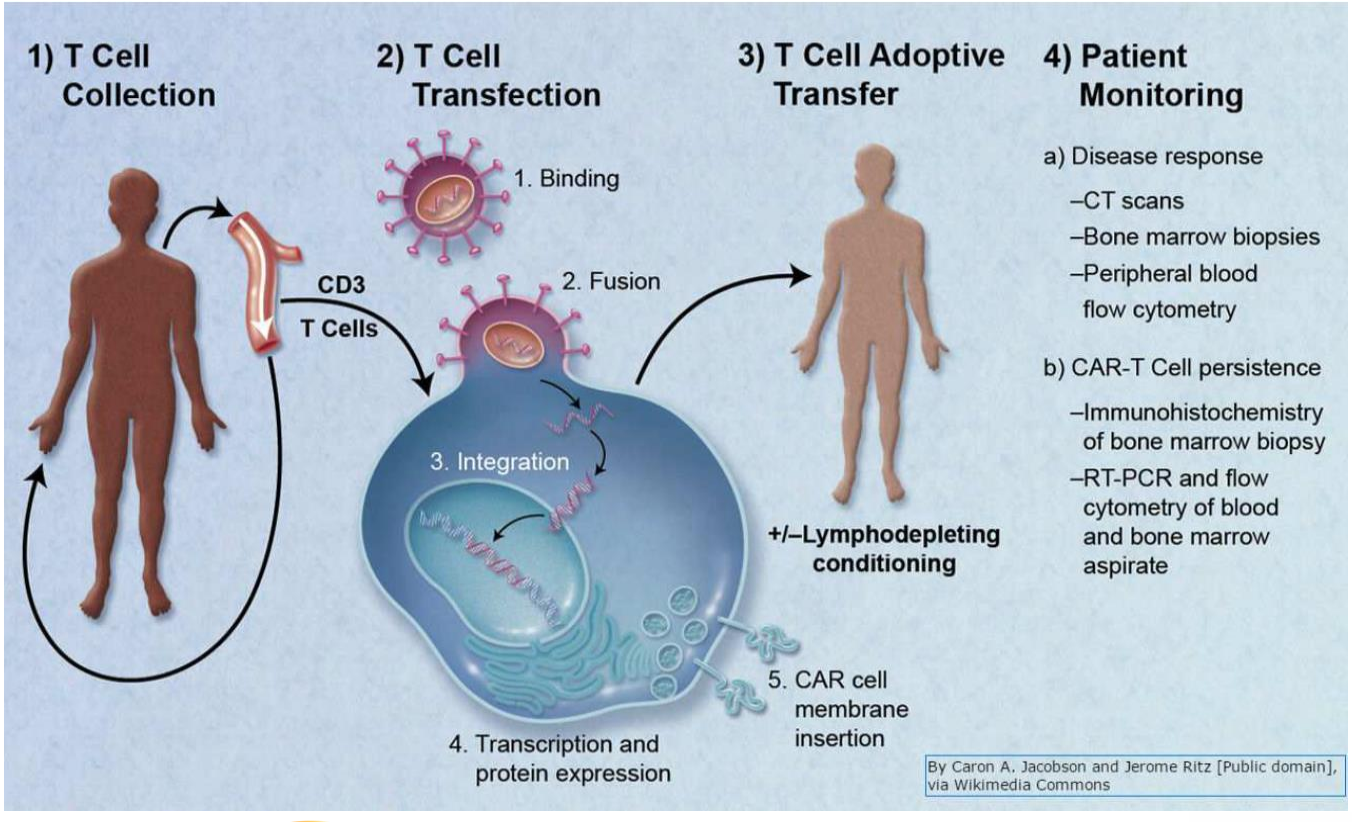
CAR-T cell therapy



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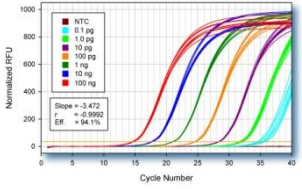
Current state of the art for cell & gene therapy detection

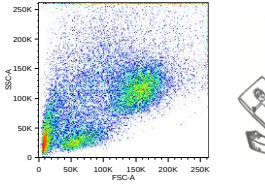
Vector presence & and transgene expression

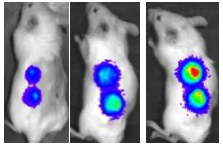
- *q-PCR*
- *RT-qPCR*
- *dd-PCR*
- *ISH*
- *IHC AQUA*

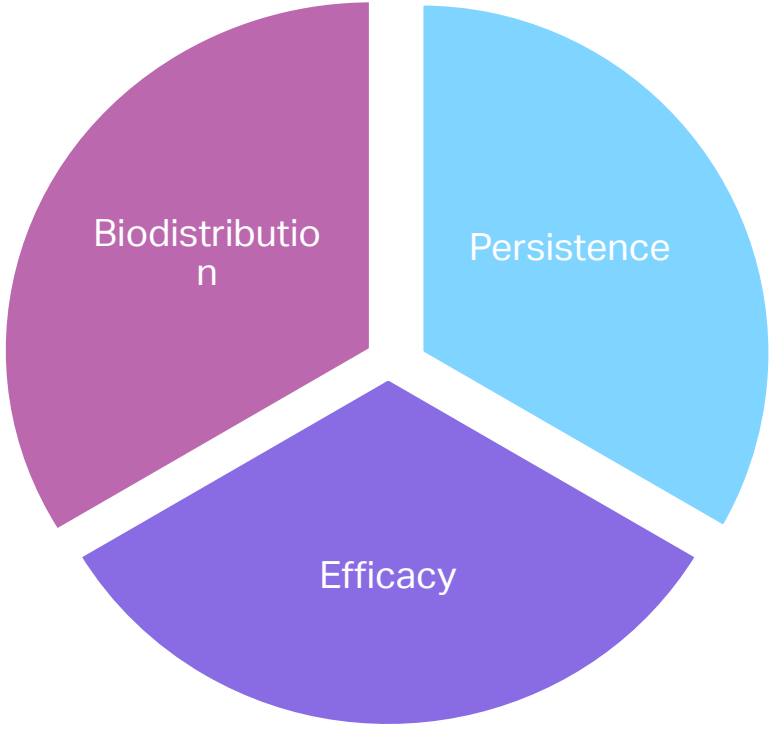


Transgene-translated protein & transduced cell detection

- *Immunohistochemistry*
- *Flow cytometry*
- *ELISA*
- *Western blot*
- *Optical imaging*







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Novel Approaches to Cell and Gene Therapy Detection

A Summary of Published Studies

AAV

- AAV Barcode-Seq - allows correlation between AAV capsid sequence and phenotype
- AAV viral vector with luciferase and DNA Barcode combination
- AAV-*bcTuD* - *AAV-expressing* barcoded Tough Decoys (stable RNA transcripts) - can be used as readouts for transduction efficiency

CAR-T

- CD8 immuno-PET – can detect T cells but does not discriminate between T cells expressing CAR and those which don't
- Human sodium iodide symporter (NIS) – high resolution CAR-T imaging but restricted due to normal tissue expression
- PET-PSMA tag - specific CAR-T cell detection but does not work for all cancer models

Quantification of transgene expression

The kinetics of CAR T migration and expansion

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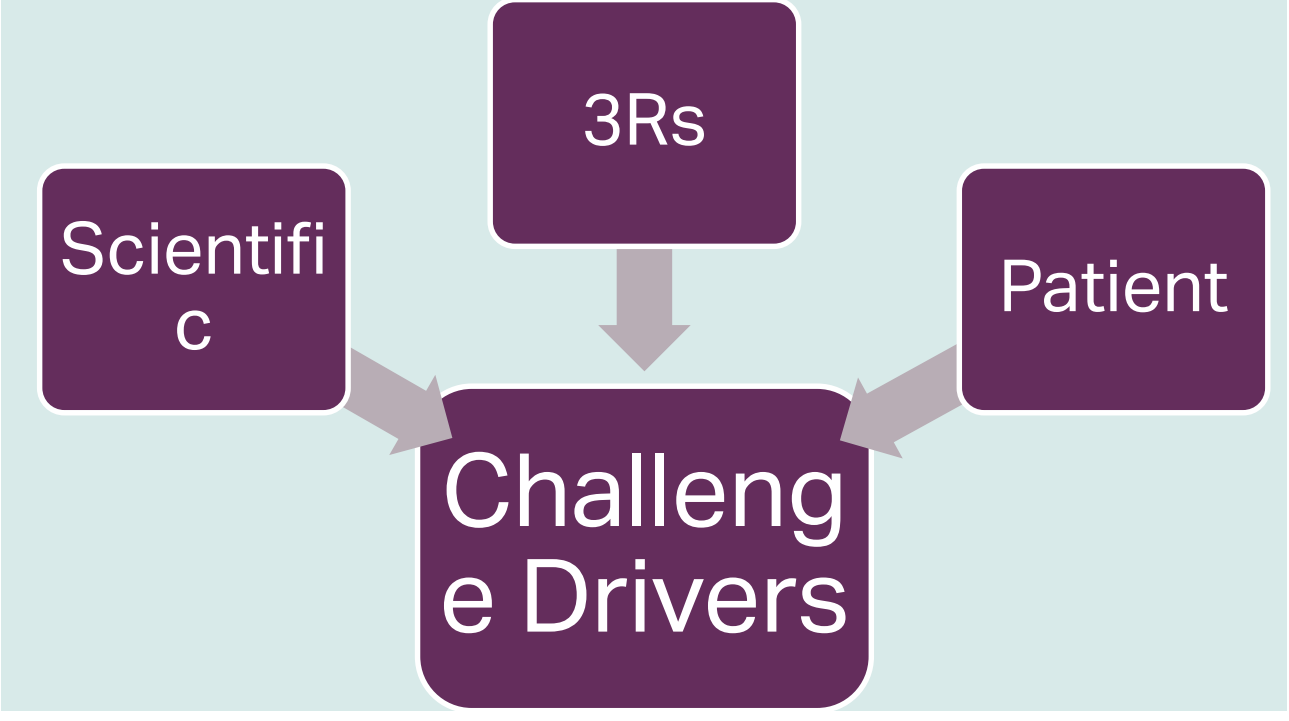
Why was this Challenge Developed?



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Patient and Scientific Benefits

- The development of a technique to monitor and quantify the kinetics of migration, biodistribution and activity of cell and gene therapy products is important for the assessment of safety risk and the development of risk mitigation strategies, contributing to the optimization of clinical safety and therapeutic outcome.
- Better selection of appropriate in vitro and animal models will improve the effectiveness and efficiency of drug development

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3Rs Benefits

Replacement

- Computational models and reagents developed during the challenge could lead to the replacement of animals and enable development of complex *in vitro* models

Refinement

- The ability to detect efficacy end-points earlier via real time imaging would reduce study duration, minimising the welfare burden on the animals

Reduction

- A sensitive *in vivo* imaging technique would allow the same animal to be followed for the whole length of a study, reducing the numbers of animals required by potentially more than 50%
- If a method to translate the biodistribution results between species was developed, the requirement to repeat the same experiments in other species would be removed

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The Challenge

Deliver a non-invasive *in vivo* imaging technique that permits the tracking of AAVs and/or CAR-T cells that will:

- Allow identification of the administered transgene across the depth of the image within the target tissue
- Provide the ability to quantify rAAV particles, transgene-transduced cells and, for CAR-T cells, measure proliferation
- Be sensitive enough to detect clusters of cells (ideally down to a lower limit of <1000 cells, if possible) or viral vectors (a copy number aligned with regulatory expectations of 50 copies of transgene per microgram of DNA)
- Demonstrate persistence and viability of the transgene-transduced cells over the time course of a study

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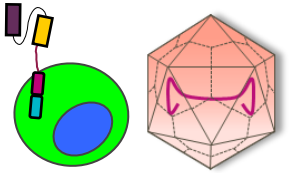
Deliverables



For Phase 1, applicants may focus on either AAVs or CAR-T cells.

Ideally, by the end of Phase 2, applicants would deliver an approach that works for both AAVs and CAR-T cells in preclinical studies and could be used in the clinical setting.
However, if it is not feasible to deliver an approach that works for both therapy types, the Sponsors are willing to focus on the development of just one.

Applicants can choose either AAV or CAR-T cells to focus on in Phase 1

All animal studies will be ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 or European Directive 2010/63/EEC and the GSK Policy on the Care, Welfare and Treatment of Animals.

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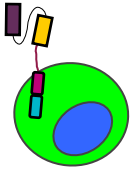
Deliverables



Phase 1 Deliverables – CAR-T

Basic *in vitro* characterisation and validation of CAR-T cell *in vivo* imaging labelling methods. Here, evidence should be provided that the label:

- Persists for longer than a month with minimal loss of sensitivity
- Is specific for transduced cells and maintains signal throughout cell proliferation

***In vitro* assessment of the developed imaging modality should include a comparison between labelled and unlabelled product, measuring the impact on the following:**

- Cell health and survival (maintenance of >70% viability throughout the study)
- Cell phenotype and proliferation kinetics
- Preliminary evidence of functionality (for example, if using labelled CAR-T cells, antigen-binding and cytotoxicity against target-expressing cells)

Pilot *in vivo* imaging data in a nude-mouse model to demonstrate label signal for up to one week

Robust plans to deliver Phase 2 of the Challenge.

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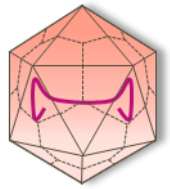
Deliverables



Phase 1 Deliverables - AAV

- Development of a reliable labelling method for real time longitudinal evaluation of rAAV cell biodistribution and quantification
- *In vitro* determination of sensitivity and comparison to classic approaches to distribution such as qPCR
- Pilot *in vivo* imaging data to demonstrate signal for up to one week with accompanying *ex vivo* data validation, using, for example, immunohistochemistry and qPCR. Preliminary evidence that the labelling procedure does not affect viral tropism, expression and toxicity characteristics of the vector
- Robust plans to deliver Phase 2 of the Challenge

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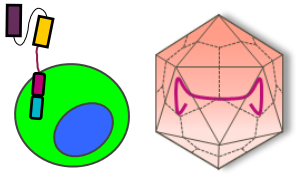
Deliverables



Phase 2 Deliverables

Full evaluation of transgene biodistribution (AAV or/and CAR-T) via a multi-modal imaging strategy that is suitable for preclinical studies

Required:

- Demonstration of the use of multimodal imaging to track both AAVs or / and CAR-T cells over several time points in a non-disease in vivo mouse model plus ex vivo validation
- Evidence of the ability to determine fluorescence intensity in three-dimensional space to precisely locate the transgene within an organ/tumour
- Reproducibility and robustness of the method(s)
- Develop an algorithm for image quantification and extrapolation to absolute T cell or vector number

Desirable:

- Mathematical model to predict T cell therapy dynamics *in vivo*
- Mathematical model to predict transgene biodistribution data in different species

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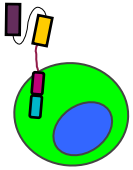
Deliverables



Phase 2 Deliverables – CAR-T

- *Ex vivo* validation of the functionality of cells after labelling. Using *in vivo* imaging techniques, this could include but is not limited to, analyses of target engagement, cytotoxicity, proliferation, quantification, survival and phenotype
- *In vivo* imaging of infused human CAR-T cells used as treatment in a human-tumour mouse xenograft model. A systematic dissection of the dynamics of CAR-T cell therapy behaviour should be made using an established algorithm to quantify the T cells in both the tumour and off-target tissues over time. This should include both short term (one week) and long-term analyses (one to three months)
- Comparison of the technique with data from immunohistochemistry, qPCR or other *ex vivo* methods of quantification from tumours or tissues

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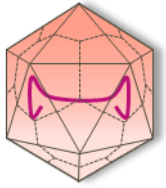
Deliverables



Phase 2 Deliverables - AAV

- *In vivo* demonstration that the labelling procedure does not affect viral tropism, expression and the toxicity characteristics
- *In vivo* determination of sensitivity and comparison to classic approaches to assess biodistribution such as qPCR. If feasible, the viral vectors should be detected at a copy number aligned with regulatory expectations of 50 copies per microgram of DNA
- Persistence of the signal (virus or transduced cells) for the entire length of the study

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What we don’t want

- Evidence of any negative impact of the imaging modality on the rAAV or CAR-T cell function will be a no-go decision point.

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Sponsor in-kind

Phase I

- Intellectual input into hypotheses development and industry perspective on applicability and impact

Phase II

- Expertise in CAR-T cell and rAAV design, development and characterisation
- Expertise in CAR-T cell efficacy mouse models
- If a successful prototype is developed, potential for Sponsor in-house testing using the system to test transferability and reproducibility of the cell and gene therapy imaging model

The provision of certain in-kind contributions may be subject to applicable legal and compliance requirements and may require prior execution of agreements.

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The human biological samples will be sourced ethically and their research use will be in accord with the terms of the informed consents under an IRB/EC approved protocol.

Thank You