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

Overall aim

The aim of this Challenge is to develop a non-invasive imaging approach to determine the *in vivo* efficacy and biodistribution of (chimeric antigen receptor) CAR-T cells and adeno-associated viruses (AAVs).

Duration

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

Budget

Phase 1: Up to £100k, Phase 2: Up to £1 million

Sponsor(s)

GSK and Novartis

Background

Cell and gene therapies are emerging treatment modalities that offer new possibilities for the treatment of previously incurable conditions. They can be broadly categorised into two subsets: *in vivo* gene therapy, which involves the introduction of a therapeutic vector directly into the patient and *ex vivo* gene therapy, which primarily uses haematopoietic cells to carry the transgenes of interest.

In vivo gene therapy using AAVs

AAV is a common delivery vector for *in vivo* gene therapy. It has not been associated with disease, is minimally immunogenic, and is able to achieve efficient and long-lived gene transfer, making it an attractive option for gene therapy applications. The number of clinical trials and marketed products based on AAVs is increasing with the percentage clinical trials involving recombinant AAVs rising from 6% to 24% in the last five years [1,2]. To date, the FDA have approved two recombinant AAVs (rAAV) for genetic diseases; *Luxturna* for the treatment of a rare form of inherited retinal disease, and *Zolgensma* for the treatment of young children with spinal muscular atrophy [3]. Currently, more than 230 clinical trials are evaluating the use of rAAV for a variety of conditions, including neurodegenerative diseases, cystic fibrosis, lysosomal storage diseases and blood disorders ((https://clinicaltrials.gov/) and (http://www.abedia.com/wiley/index.html)).

Ex vivo gene therapy using CAR-T cells

Clinical trials for the treatment of cancer have used a number of different *ex vivo* gene therapy strategies to target the tumour. The modality that has advanced furthest in clinical development is CAR-T cell therapy. Using this approach, patient T cells are genetically engineered to express CARs, which are molecules containing a binding domain specific to the target tumour.

Companies including Novartis and GSK, are evolving new CAR-T therapies targeting different antigens specific for various types of blood cancers and solid tumours. In 2017, the FDA approved the first two CD19 CAR-T cell therapies — Kymriah and YESCARTA.

These landmark approvals have spurred major development in the field, with more than 400 CAR-T cell therapies currently being investigated in Phase I-III clinical trials worldwide (https://clinicaltrials.gov/). The market is anticipated to triple in size, reaching over $8.21 Billion by 2025 [4], making it the fastest growing sector in the regenerative medicine industry.

Assessment of safety and disposition

Not all conventional pharmacokinetic parameters (e.g. absorption, distribution, metabolism and excretion) apply to gene therapy. The disposition and persistence of the therapeutic product should be understood prior to first dose in humans as even a single dose of a gene therapy product could persist in the body for a long time (potentially for life). These data may also influence the design of preclinical safety studies (e.g. dose, duration, timepoints etc). In addition, for viral vectors, the possibility of inadvertent germline transmission (vertical transmission) or of shedding (dissemination of the vector into the environment) — with the risk of infection of healthcare professionals or patient's family (horizontal transmission) — must be addressed. Other potential toxicities can include integration site specific hazards, immuno-toxicity and for CAR-T cells, acute on-target, off-tumour [5], off-target, off-tumour [6,7] and neurological toxicities [8,9,10]. The design of the biodistribution studies, as well as the bioanalytical techniques used, will differ based upon the objective of the study.

Immunohistochemical and PCR techniques have been applied to determine the biodistribution, persistence and shedding of gene therapy products into tissues and tumour sites [11,12]. However, they require the animals to be euthanised. These analyses also often demonstrate significant heterogeneity and do not provide information on the temporal and spatial localisation of the gene therapy, both within the target tissue and other organs.

Novel approaches that have been developed for biodistribution studies for AAVs include:

- AAV Barcode-Seq — a novel method that allows the collection of high-throughput data on the correlation between AAV capsid sequence and phenotype, with the potential to monitor the biodistribution of several viruses at the same time [13].

- A new AAV vector coding a firefly luciferase and a fluorescent protein in combination with a DNA barcode to optimise data readouts from *in vivo* studies [14]. The presence of the luciferase reporter allows for longitudinal bioluminescent imaging studies, while the use of barcoded capsid variants offers the possibility to track several viruses in the same animal.

- AAV-*bcTuD, an AAV expressing* barcoded Tough Decoys (*bcTuD*), which are highly stable RNA transcripts that can be used as readouts for transduction efficiency [15].

A major limitation of these methods is the potential competition among capsid variants for cell receptors that might modify the performance of viruses when used alone. Moreover, the signal from the firefly luciferase used for the *in vivo* imaging shows low penetration depth and poor quantification capabilities, limiting its use.

Magnetic resonance imaging possesses several advantages that make it suitable for cellular imaging, such as superior soft tissue contrast and high resolution, but so far has only been used for local and not systemic biodistribution of rAAV [16, 17].

Approaches that have been developed to track CAR-T cells include:

- High contrast immuno-positron emission tomography (immuno-PET) detecting CD8 protein has previously shown efficacy for tracking the biodistribution of T cells *in vivo* [18,19]. While this approach is useful to detect the total CD8 positive cell population, it does not discriminate between cells that have been transduced by the therapy and those that have not.

- The human sodium iodide symporter (NIS) system for specific high-resolution serial imaging of CAR-T cells, *in vivo* [20]. This technique has potential for translation to the clinic but is restricted because several normal tissues also express the NIS protein.

- *Ex vivo* labelling of CAR T cells using isotopes (e.g. 89Zr-oxine, In-111 oxine). This also labels the total cellular population and due to isotope half-life, limits the determination of only the initial biodistribution over the first seven days post-dosing [21].

- A recent study used PET tracing to detect prostate-specific membrane antigen (PSMA)-tagged CD19 CAR-T cells in a mouse model of acute lymphoblastic leukemia. Although this approach is a significant step forward in terms of CAR-detection this approach cannot be applied to all cancer models and further work is required to quantify the kinetics of CAR T migration and expansion [22].

The development of a technique to monitor and quantify the kinetics of migration, biodistribution and activity of cell and gene therapy products is critical for both the elucidation of therapeutic efficacy and safety, and to minimise the risk of attrition in clinical trials.

3Rs benefits

The gold standard preclinical *in vivo* study to assess gene therapy biodistribution, involves necropsy and time-consuming immunohistochemical or molecular analysis of numerous tissue sections or homogenates.

A preclinical viral vector biodistribution study is a core regulatory requirement prior to initiating a clinical trial, and has been recently described in the FDA, EMA and cross-health authority publications [23,24,25]. A pilot experiment for the evaluation of one viral vector with three time points and one species would require the use of 15 animals. However, to meet the current regulatory expectations, an average of 40-60 animals are required per study.

At present, there is no regulatory requirement to track the biodistribution of CAR-T cells, but it is desirable to obtain information regarding the effective targeting of the cells to the tumour and the efficacy and persistence of the cells. At the pre-candidate selection stage, GSK runs approximately four studies per year. As an example, in a human tumour xenograft efficacy study in mice, a CAR construct would be evaluated at two different cell doses plus controls, using up to eight animals per group per gender. In order to fully evaluate the dynamics of T cell behaviour, even over a short time period, cohorts of animals would be culled at at least two time points, resulting in 96 animals being used.

A sensitive *in vivo* imaging technique developed through this Challenge would mean that the same animal could be followed for the whole length of a study, reducing the numbers of animals required by potentially more than 50%. The ability to detect efficacy end points earlier would also reduce study duration, minimising the welfare burden on the animals and deliver cost efficiencies.

In addition, if a reliable method to translate the biodistribution results between species was developed, the requirement to sometimes repeat the same experiments in other species would be removed.

Key deliverables

Essential:

The overall deliverable for this Challenge is to develop a non-invasive *in vivo* imaging technique that permits the tracking of AAVs and CAR-T cells. The technique developed should:

- Permit tracking of the transgene to target and off-target sites within the organism.

- Allow identification of the administered transgene across the depth of the image within the target tissue.

- Provide the ability to quantify transduced cells, and for CAR-T cells, measure proliferation.

- Demonstrate that any alteration to the CAR or AAV architecture does not affect transduction and function.

- Allow imaging to take place over multiple time-points (e.g. weekly for one month and then monthly, for up to three months).

- 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 per microgram of DNA).

- Demonstrate persistence and viability of the transgene-transduced cells over the time course of the study.

- Not increase the welfare burden on the experimental animals (e.g. by requiring the animals to be shaved). Nude mice are appropriate for most investigations, unless otherwise stated.

Desirable:

- The approach is applicable to multiple preclinical species.

- The potential for translation to use in the clinic.

For Phase 1, applicants may focus on either AAVs or CAR-T cells. Ideally, by the end of Phase 2, applicants should deliver an approach that works for both AAVs and CAR-T cells. 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.

Phase 1 Deliverables

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

For a CAR-T cell-based Phase 1 project:

- Basic *in vitro* characterisation and validation of CAR-T cell labelling using methods suitable for real time *in vivo* imaging (i.e. optical or molecular imaging). Here, evidence should be provided that the label:

    - Persists for longer than a month with minimal loss of sensitivity.
    - Is specific for transduced cells.
    - Maintains signal throughout proliferation of the transduced cells.

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

- *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.

        - Proliferation kinetics.
        - Antigen-binding and cytotoxicity against target-expressing cells (if using labelled CAR-T cells).

- Demonstrate reproducibility between *in vitro* studies.

- Pilot *in vivo* imaging data in a nude mouse model to demonstrate label signal for up to one week with accompanying *ex vivo* data validation using immunohistochemistry of target and non-target tissues and phenotyping of engrafted CAR-T cells.

- Robust plans to deliver Phase 2 of the Challenge.

For an AAV-based Phase 1 project:

- Development of a reliable labelling method for real time longitudinal evaluation of rAAVs cell biodistribution and quantification.

- *In vitro d*etermination of sensitivity and comparison to classic approaches to distribution such as qPCR.

- Evidence of any negative impact of the imaging modality on the rAAVs will be a no-go decision point.

- 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 potential toxicity of the vector.

- Robust plans to deliver Phase 2 of the Challenge.

Phase 2 Deliverables

Full evaluation of transgene biodistribution (AAV 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 and CAR-T cells over several time points in a non-disease *in vivo* mouse model.

- *Ex vivo* evaluation of the gene and protein expression profile of the transgene-transduced cells.

- Evidence of the ability to determine fluorescence intensity in three-dimensional space to precisely locate the transgene within an organ/tumour.

- Reproducibility between different labelling studies (and different T cell donors for CAR-T cells).

- Validated algorithm for image quantification and extrapolation to absolute T cell or vector number.

- Data to demonstrate applicability of the suggested approach to multiple gene therapy constructs or other cell types.

For CAR-T cells:

- *Ex vivo* validation of the *in vivo* imaging technique in terms of T cell therapy behaviour. 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 (preferably solid cancer) nude mouse xenograft model. A systematic dissection of the dynamics of CAR-T cell therapy behaviour should be made using a validated 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).
- Validation of the technique with data from immunohistochemistry or other *ex vivo* methods of quantification from tumours or tissues.

For AAVs:

- *In vivo* demonstration that the labelling procedures does not affect viral tropism, expression and potential toxicity.
- *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 for the entire length of the study.

<u>Desirable</u>

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

It is important to note that the CRACK IT Challenges competition is designed to support the development of new 3Rs technologies and approaches, which will improve business processes and/or lead to new marketable products. The applications for both Phases must include a plan to commercialise the results into a product or service. This should be taken into consideration when completing your application.

Sponsor in-kind contributions

The Sponsors will provide:

- Access to CAR and AAV viral vectors as appropriate.
- Access to preclinical and clinical samples as appropriate.
- Expertise in CAR-T cell and rAAVs design, development and characterisation.
- Expertise in CAR-T cell efficacy mouse models.
- Regular scientific advice on model development, data analysis, and data interpretation.
- 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.

References

1. Hanna E *et al.* (2017). Gene therapies development: slow progress and promising prospect. *J Mark Access Health Policy,* 5**,** 1265293.

2. Goswami R *et al*. (2019). Gene Therapy Leaves a Vicious Cycle. *Front Oncol,* 9, 297.

3. FDA Website (2019), *Approved Cellular and Gene Therapy Products* [Online] Available at: https://www.fda.gov/vaccines-blood-biologics/cellular-gene-therapy-products/approved-cellular-and-gene-therapy-products. [Accessed: 2 July 2019].

4. Biopharma Reporter Website (2019), *Cell Therapy market to triple in size by 2025* [Online] Available at: https://www.biopharma-reporter.com/Article/2019/03/04/Cell-therapy-market-to-triple-in-size-by-2025. [Accessed: 2 July 2019].

5. Morgan RA *et al*. (2010). Case report of a serious adverse event following the administration of T cells transduced with a chimeric antigen receptor recognizing ERBB2. *Mol Ther*, 18, 843-51.

6. Cameron BJ *et al*. (2013). Identification of a Titin-derived HLA-A1-presented peptide as a cross-reactive target for engineered MAGE A3-directed T cells. *Sci Transl Med*, 5, 197re103.

7. Linette GP *et al.* (2013). Cardiovascular toxicity and titin cross-reactivity of affinity-enhanced T cells in myeloma and melanoma. *Blood,* 122, 863-71.

8. Maude SL *et al.* (2014). Chimeric antigen receptor T cells for sustained remissions in leukemia. *N Engl J Med*, 371, 1507-17.

9. Davila ML *et al.* (2014). Efficacy and toxicity management of 19-28z CAR-T cell therapy in B cell acute lymphoblastic leukemia. *Sci Transl Med*, 6, 224ra25.

10. Lee DW *et al.* (2015). T cells expressing CD19 chimeric antigen receptors for acute lymphoblastic leukaemia in children and young adults: a phase 1 dose-escalation trial. *Lancet*, 385, 517-528.

11. Brentjens R *et al*. (2010). Treatment of chronic lymphocytic leukemia with genetically targeted autologous T cells: case report of an unforeseen adverse event in a phase I clinical trial. *Mol Ther*, 18, 666-8.

12. Porter DL *et al.* (2011). Chimeric antigen receptor-modified T cells in chronic lymphoid leukemia. *N Engl J Med,* 365, 725-33.

13. Adachi K *et al.* (2014). Drawing a high-resolution functional map of adeno-associated virus capsid by massively parallel sequencing. *Nat Commun* 5, 3075.

14. Marsic D, Mendez-Gomez HR and Zolotukhin S (2015). High-accuracy biodistribution analysis of adeno-associated virus variants by double barcode sequencing. *Mol Ther Methods Clin Dev*, 2, 15041.

15. Xu M *et al.* (2019) High-Throughput Quantification of *In Vivo* Adeno-Associated Virus Transduction with Barcoded Non-Coding RNAs. *Human Gene Therapy* (Epub ahead of print). doi.org/10.1089/hum.2018.253.

16. Meier S *et al.* (2018) Non-invasive detection of adeno-associated viral gene transfer using a genetically encoded CEST-MRI reporter gene in the murine heart. *Scientific Reports* 8:4638.

17. Ohno K, *et al.* (2019) Kinetics and MR-Based Monitoring of AAV9 Vector Delivery into Cerebrospinal Fluid of Nonhuman Primates. *Mol Ther Methods Clin Dev*. 8 (13):47-54.

18. Tavare R *et al*. (2016). An Effective Immuno-PET Imaging Method to Monitor CD8-Dependent Responses to Immunotherapy. *Cancer Res*, 76, 73-82.

19. Tavare R *et al.* (2014). Engineered antibody fragments for immuno-PET imaging of endogenous CD8+ T cells *in vivo. Proc Natl Acad Sci USA,* 111, 1108-13.

20. Emami-Shahri N *et al.* (2018). Clinically compliant spatial and temporal imaging of chimeric antigen receptor T-cells. *Nat Commun*, 9, 1081.

21. Weist MR *et al.* (2018) PET of Adoptively Transferred Chimeric Antigen Receptor T Cells with 89Zr-Oxine. *J Nucl Med.* 59(10):1531-1537.

22. Minn Il *et al.* (2019) Imaging CAR T cell therapy with PSMA-targeted positron emission tomography. *Science Advances,* 5:eaaw5096.

23. FDA Website (2018), *Long Term Follow-up After Administration of Human Gene Therapy Products* [Online] Available at: https://www.fda.gov/regulatory-information/search-fda-guidance-documents/long-term-follow-after-administration-human-gene-therapy-products FDA-2018-D-2173. [Accessed: 2 July 2019].

24. European Medicines Agency Website (2008), *Guideline on safety and efficacy follow-up and risk management of advanced therapy medicinal products* [Online] Available at: https://www.ema.europa.eu/en/guideline-safety-efficacy-follow-risk-management-advanced-therapy-medicinal-products EMEA/149995/2008. [Accessed: 2 July 2019].

25. IPRP Website (2018), *Reflection paper- General Principles to Address the Nature and Duration of Follow-up for Subjects of Clinical Trials Using Cell Therapy Products* [Online] Available at: http://development.iprp.backend.dev6.penceo.com/sites/default/files/2018-12/IPRP_CTWG_ReflectionPaper_2018_1017.pdf**.** [Accessed: 2 July 2019].