



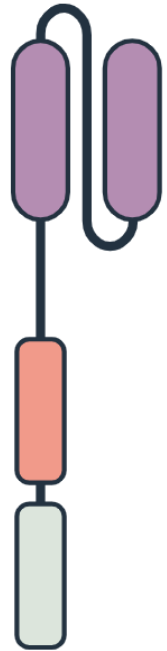
CAR-T

Design, engineering,
production and
characterisation
capabilities

hello@rouken.bio



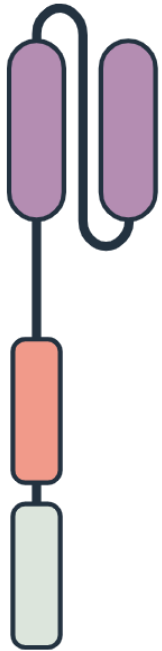
Cell therapy: tools and competencies



Chimeric antigen receptor (CAR) T cell production

- **Construct design:** utilisation of in-house CAR-T stocks or our molecular biologists can design CAR constructs to custom specifications
- **Cells:** Desired T cell population purification +/- unwanted cell removal steps
- **Engineering:** Lentiviral production and transduction
- **Production:** T cell expansion protocol using G-Rex® technology, for scaled down version of a typical GMP-manufacturing process

Cell therapy: tools and competencies



CAR T cell production - Product characterisation

Product specification: determine fold expansion, cellular viability, % CAR positivity and cellular composition (lineage) by flow cytometry

- **Functionality:** cytotoxic potential by non-invasive kinetic measurements by xCELLigence RTCA and endpoint assays; flow-based (viability, cell count, apoptosis, proliferation *etc.*), plate-based (luminescence and colorimetric) and even CAR-T reporter cell assays. Measure cellular avidity *via* our partners.
- **Extended immunophenotyping:** advanced multi-parameter flow cytometry to analyse cell sub-populations [effector and memory], exhaustion markers, activation markers and cytokine production.

⊕ Case study 1

Her2 CAR-T

- **STEP 1** – Panel of Her2 targeting CAR constructs design from publicly available sequences and lentiviral (LV) production
- **STEP 2** – Isolation of pan CD3 T cells from PBMCs > Expansion with α -CD3/CD28 + IL-2 > transduction with LV > re-feed to promote *in vitro* expansion
- **STEP 3** – Target cell creation – ‘Blank canvas’ CHO cells engineered to express variable levels of target antigen
- **STEP 4** – Characterisation of cytotoxic capacity using kinetic measurements



Case study 1

Comparing high and low affinity HER2 targeting CAR-Ts

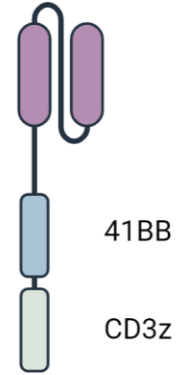
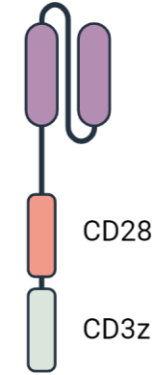
- **STEP 1 – CAR design**

- 4 CAR designs
 - 3rd generation
 - Varied co-stimulatory, 'signal 2' domains; 4-1BB or CD28
 - Varied scFv affinity to Her2
 - Common activation, 'signal 1' domain; CD3 zeta

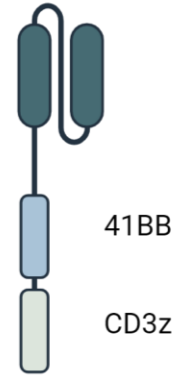
- **STEP 2 – CAR-T cell production**

- Lentivirus from each CAR used to transduce expanding CD3+ T cells from healthy human donors
- Expanded CAR-T cells cryopreserved

High Affinity scFv



Low Affinity scFv

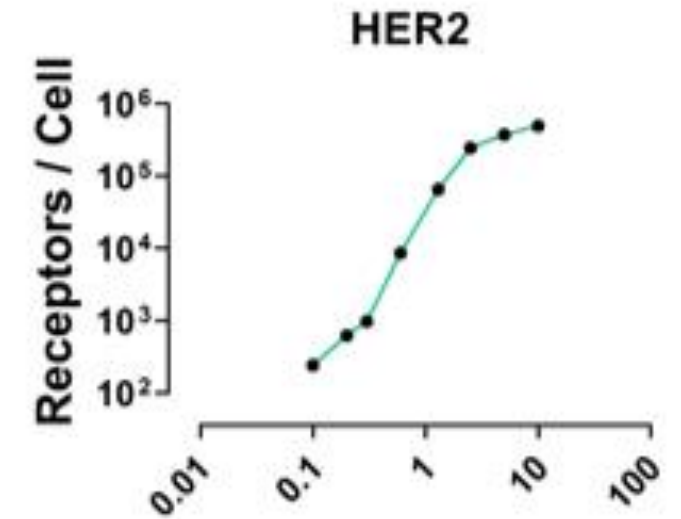
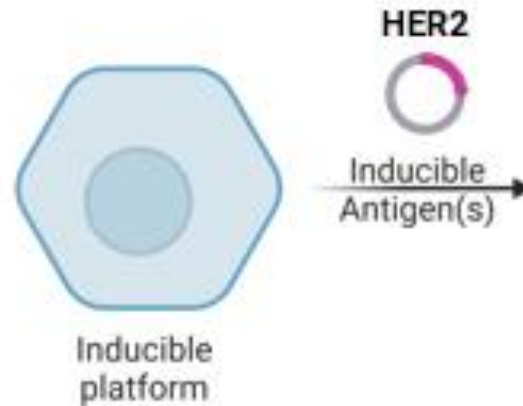




Case study 1

Comparing high and low affinity HER2 targeting CAR-Ts

- **STEP 3** – Target cell line creation
- CHO-Her2 cells with inducible levels of Her2
- Utilisation of proprietary IndEx-2 platform to create cell line with tuneable Her2 expression

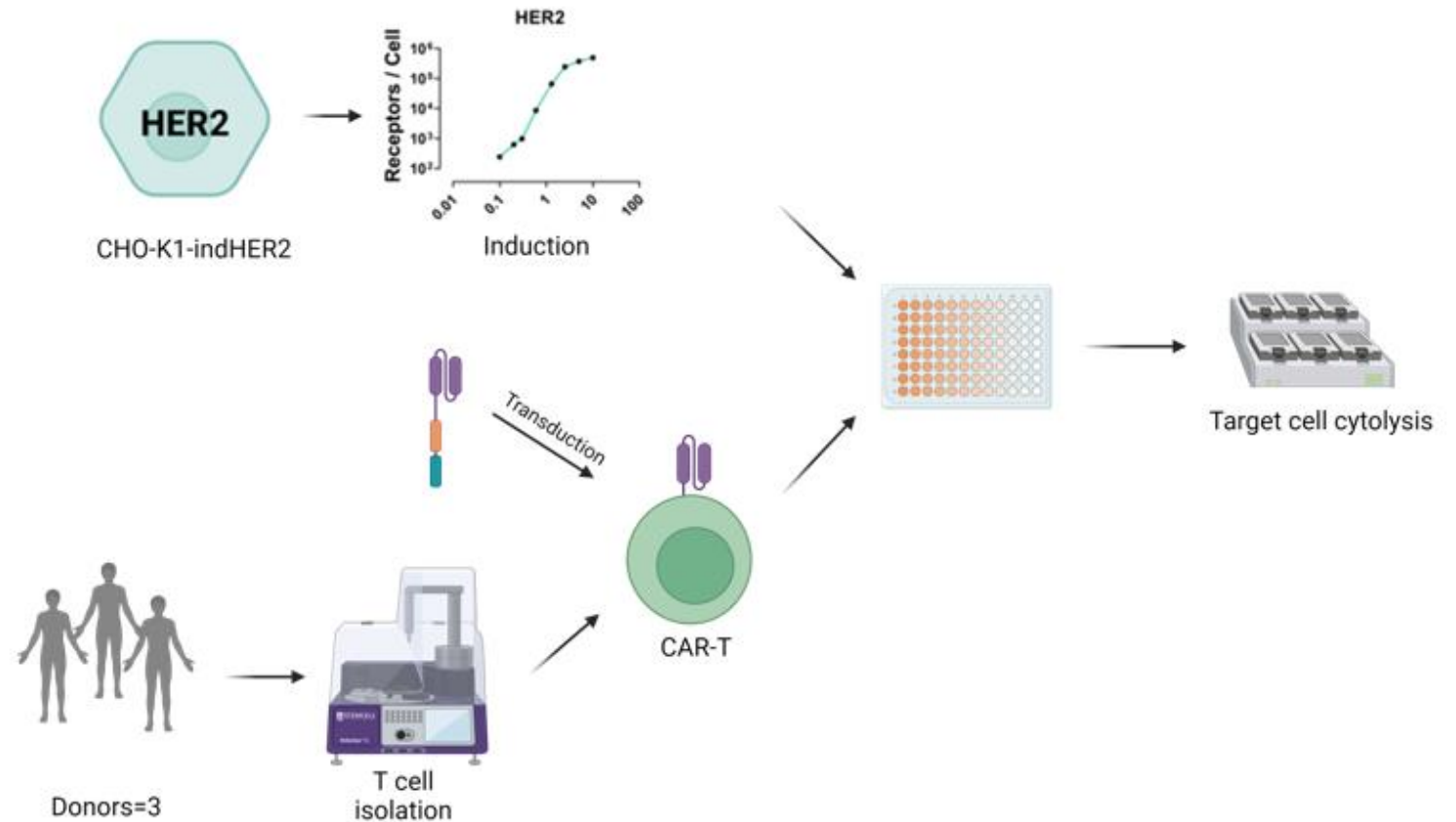




Case study 1

Comparing high and low affinity HER2 targeting CAR-Ts

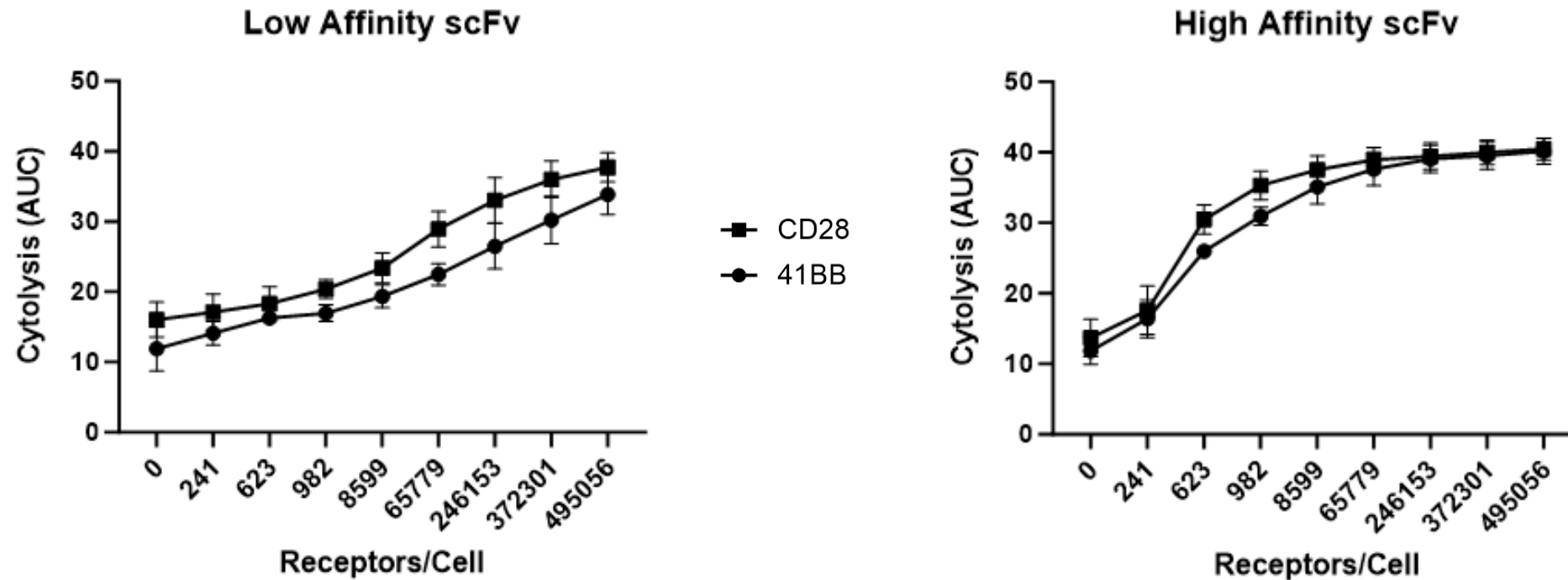
- **STEP 4**
- Assay set up for the characterisation of cytotoxic capacity using kinetic measurements





Case study 1

Comparing high and low affinity HER2 targeting CAR-Ts



⊕ Case study 2

Dual CAR-T

- **STEP 1** – Design of CD19 and CD20 ‘OR GATE’ CAR construct
- **STEP 2** – Transduction of Jurakt-NFAT-Luc reporter cells with LV
- **STEP 3** – Target cell creation – ‘Blank canvas’ CHO-K1 cells engineered to express CD19 and CD20 under distinct inducers for independent induction of each
- **STEP 4** – Characterisation of cytotoxic capacity using luminescence (reporter cell)

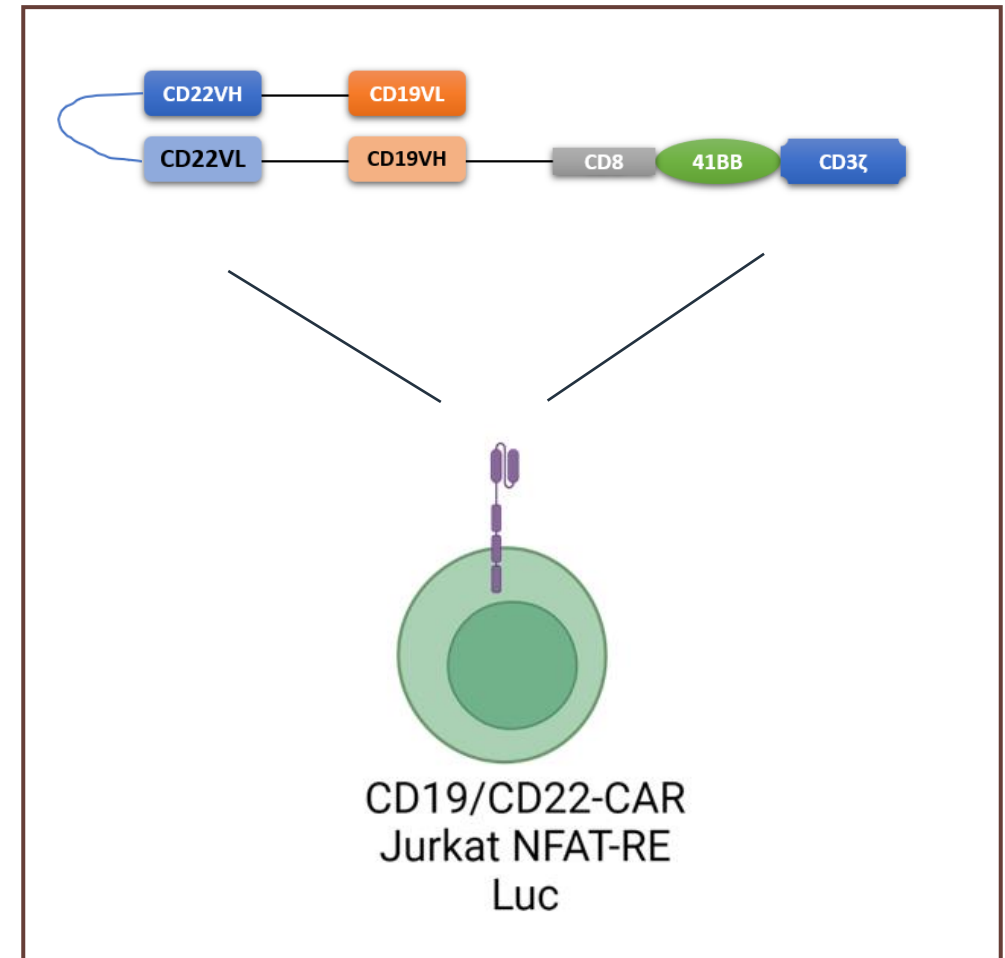




Case study 2

Dual CAR-T

- **STEP 1** – Design of CD19 and CD20 ‘OR GATE’ CAR construct
- **STEP 2** – Transduction of Jurakt-NFAT-Luc reporter cells with LV

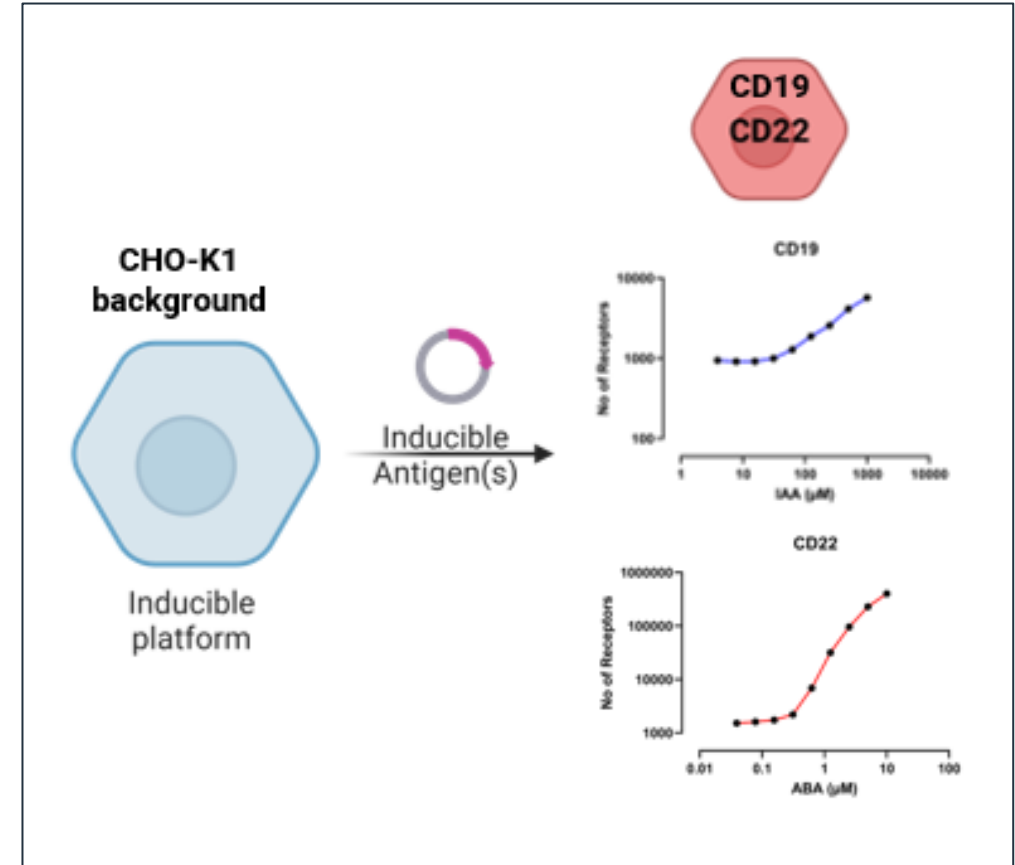




Case study 2

Dual CAR-T

- **STEP 3** – Target cell line creation
- CHO-CD19/CD22 cells with inducible levels of each
- Utilisation of proprietary IndEx-2 platform to create cell line with independent tuneable expression of CD19 and CD22

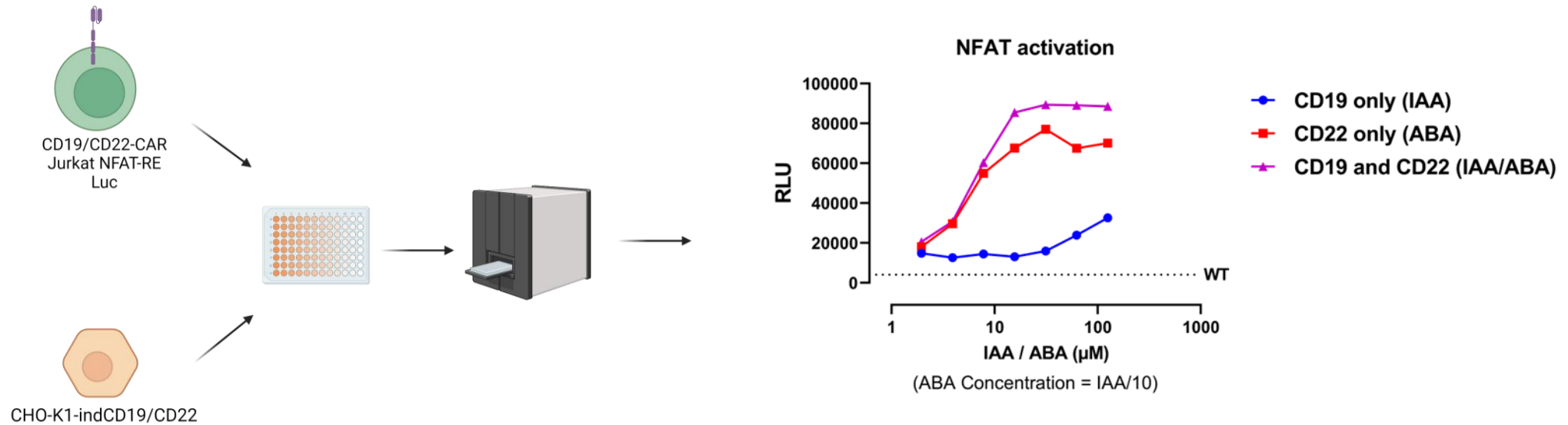




Case study 2

Dual CAR-T

- STEP 4** – Characterisation of cytotoxic capacity using luminescence (reporter cell)





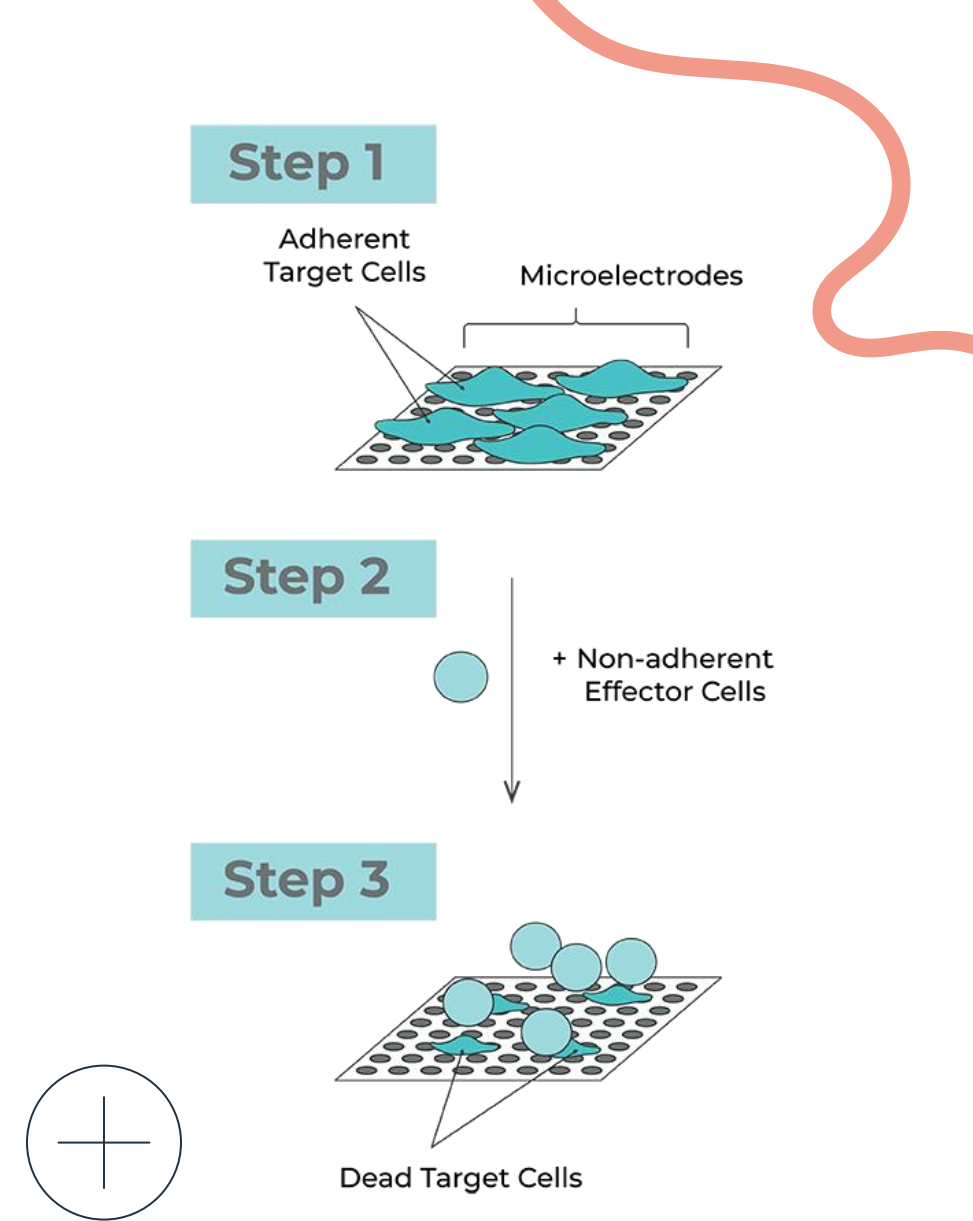
Appendices

⊕ In house stocks of CAR-T tools

CAR design		In house availability	
scFv	Signalling domain	Lentiviral stocks	Cryopreserved cells
Her2 (high affinity)	2 nd Gen: 4-1BB, CD3zeta	Yes	Yes – primary T cells
	2 nd Gen: CD28, CD3zeta	Yes	Yes – primary T cells
Her2 (low affinity)	2 nd Gen: 4-1BB, CD3zeta	Yes	Yes – primary T cells
	2 nd Gen: CD28, CD3zeta	Yes	Yes – primary T cells
CD19 (FMC63)	2 nd Gen: CD28, CD3zeta	Yes	Yes – primary T cells
CD19 & CD22 (dual)	2 nd Gen: CD28, CD3zeta	Yes	Yes – Jurkat NFAT reporter cells

⊕ Agilent xCELLigence RTCA System

- Label free methodology
- Simple
- Continuous measurement
 - Kinetic data
- Can be combined with other readouts
 - Flow cytometry and cytokine analysis
- We employ it for:
 - mAbs (ADCC)
 - Immune cell engagers
 - CAR-T and TCR-T
 - ADCs





Speak to our experts to accelerate your drug discovery project with our CAR-T services

Backed by our brilliant minds, we have a strong track record of designing, engineering, producing and characterising CAR-T solutions to custom specifications.

Our molecular biologists and immunologists work hand-in-hand with you to create the most appropriate system tailored to your unique goals.



www.rouken.bio



hello@rouken.bio

