Personalised tumour-trained lymphocytes derived from regional lymph nodes for treatment of colorectal cancer

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Introduction

Adoptive T cell therapy has gained increasing interest in cancer therapy. We present a novel personalized tumour-draining lymph node-derived T cell therapy targeting tumour neoantigens: **personalized Tumour-Trained Lymphocytes (pTTL)**.

pTTL intend to **re-educate** immune cells to **eradicate** tumour cells **regardless of cancer type**.

pTTL consists of autologuous regional lymph nodes (RLN) **tumour-antigen selected T-cells**.

A First-in Human trial of pTTL is underway. The indication for this trial is **colorectal cancer**, a common cancer with poor prognosis in advanced stage disease.

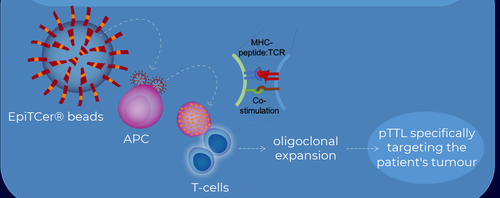
Highlights

- pTTL can be applied in any cancer expressing targetable neoantigens by the use of **PIOR® Manufacturing**.
- Personalised cancer immunotherapy therapy is tailored for the patient's own tumour and takes advantage of the patient's immune system.
- The **EpiTCer® technology** offers a novel approach for efficient antigen delivery and specific T cell activation. Also applicable to the use of alternative starting material.
- pTTLs are produced in a controlled and reproducible GMP manufacturing process.
- pTTLs are part of a virtuous cycle: every production linked to patient's tumour characteristics provides information to develop both process and product further.

Regional lymph nodes as starting material

Response to stimulation/tumour antigens is more efficient compared to non-draining lymph nodes, blood or tumour-infiltrating lymphocytes.

pTTL tumour antigen selection and expansion



PIOR® Manufacturing and the EpiTCer® technology

neoantigen identification, ranking, production and delivery

1. The right target find/rank

2. Production design/coupling

3. Efficient delivery phagocytosis/MHC-presentation

EpiTCer beads:
3-6 neoantigen proteins containing 6 epitopes each coupled to paramagnetic beads

PIOR® Manufacturing → EpiTCer® technology

PIOR® Manufacturing is a software for identification, selection and ranking developed by NEOGAP Therapeutics. Design of neoantigen constructs for EpiTCer® beads is also performed by PIOR® Manufacturing.

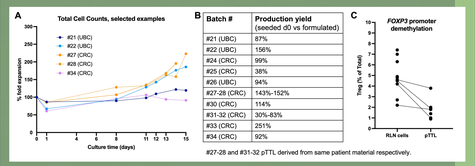
EpiTCer® beads are specially developed to promote efficient phagocytosis, neoantigen processing and presentation by APCs. The bead size, 1 µm, promotes phagocytosis. The process employs natural antigen processing, is **HLA agnostic** and **promotes cross-presentation**. The paramagnetic nature of the beads allows sterilization and robotic processing compatible with large scale GMP production and enables removal of the beads from the cell therapy product.

pTTL characterisation

The characterisation of n=12 pTTL development batches derived from urinary bladder cancer (UBC) or colorectal cancer (CRC) is presented.

Expansion data

- Patient's individual variations confer growth kinetic diversity.
- pTTLs derived from UBC or CRC patient material show comparability.
- Distinct pTTL production from same starting material batch show consistency.
- EpiTCer® bead stimulation does not favour regulatory T cell expansion.

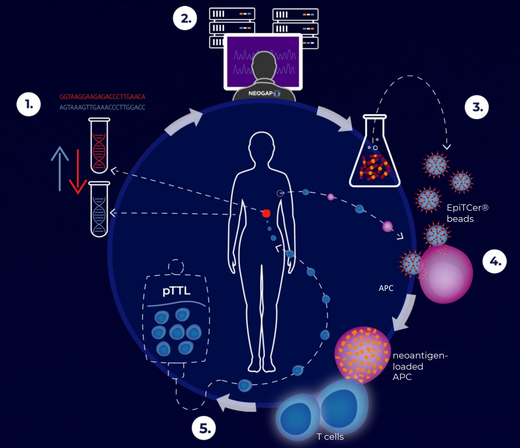


Batch #	Production yield (seeded d0 vs formulated)
#21 (UBC)	87%
#22 (UBC)	156%
#24 (CRC)	99%
#25 (CRC)	38%
#26 (UBC)	94%
#27-28 (CRC)	143%-152%
#30 (CRC)	114%
#31-32 (CRC)	30%-63%
#33 (CRC)	251%
#34 (CRC)	92%

#27-28 and #31-32 pTTL derived from same patient material respectively.

(A) Selected examples of pTTL culture growth kinetics. (B) Summary of obtained production yields. (C) Proportion of bona fide regulatory T cells before (RLN cells) and after culture (pTTL) by analysis of *FOXP3* promoter demethylation (PureQuant™ Treg assay).

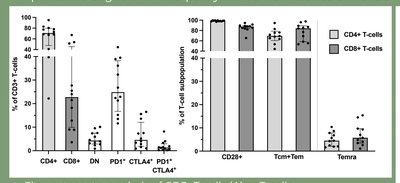
pTTL production process overview



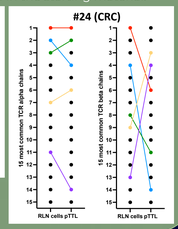
1. Collection of tumour material and peripheral blood samples for next generation sequencing (NGS).
2. Analysis of NGS data by in house software system PIOR® Manufacturing for neoantigen identification, selection and ranking.
3. **Production of EpiTCer® beads**, including coupling of neoantigens to super-paramagnetic beads.
GMP-production
4 (GMP). Surgical collection of RLNs and ***in vitro* culture** with EpiTCer® beads for pTTL expansion.
5.(GMP). Cell harvest and **pTTL formulation**. pTTL product is infused to the patient.

pTTL identity

- Patient's individual variation confers pTTL's phenotypic diversity (CD4⁺ and CD8⁺ T-cells).
- pTTLs are mainly composed of Tcm or Tem cells. Only a small proportion display a phenotype indicative of a limited *in vivo* functionality.
- pTTLs are oligoclonal and partly derive from clones enriched in the starting material.



Flow cytometry analysis of CD3⁺ T-cells (A) or T-cells subpopulations (B). DN: CD3⁺CD4⁻CD8⁻ T-cells, Tcm+Tem: CD45RA⁻CCR7⁺/⁻, Temra: CD45RA⁺CCR7⁻. (C) Conservation of the 15 most common TCR chains between RLN cells and pTTLs.



Clinical study design

Phase I/II First-in-Human Trial

Up to 16 (goal 12 evaluable) adult patients with **colorectal cancer Stage IV** :
- have received available "standard of care" (SoC) Or for whom SoC is not in their best interest Or that are in a scheduled pause in SoC
- have measurable disease (RECIST1.1)
- present with a minimum life expectancy of 3 months at treatment

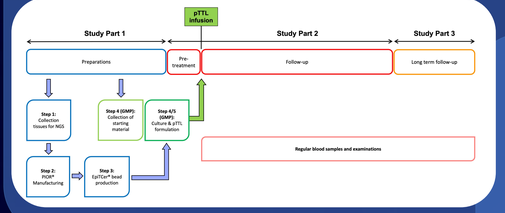
Endpoints

Primary endpoint: safety

Secondary endpoints: standard clinical measurements:
- response
- OS, PFS, disease-specific survival
- time to progression

Exploratory endpoints:
- Biomarker analyses
- pTTL *in vivo* characterisation

Study design



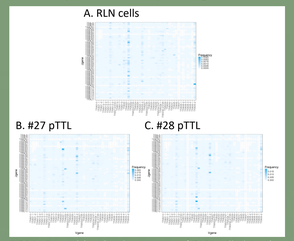
Part I
- Collection of tumour material and blood for NGS
- Collection of regional lymph nodes (abdominal surgery)
- Production of pTTL

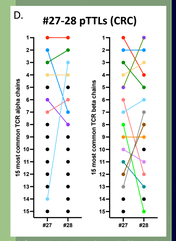
Part II
- Pre-conditioning
- pTTL administration
  Dose escalation in 4 cohorts
- Follow-up

Part III
- Long-term follow up

pTTL specificity

Comparing batches derived from the same starting material shows that the pattern of TCR alpha V-J combinations is very similar between both products with some overlap with the starting material and supports personalised EpiTCer® bead specificity.





TRA V-J combination heatmaps of RLN cells (starting material from CRC patient (A)) and derived pTTLs (#27 (B) and #28 (C)). Conservation of the 15 most common TCR chains in pTTL batches #27 or #28, derived from the same material (D).

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