A first in human Phase I/IIa trial of personalised Tumour-Trained Lymphocytes, pTTL, derived from regional lymph nodes for treatment of colorectal cancer

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Introduction

pTTL (personalised Tumour-Trained Lymphocytes) is a novel adoptive T cell therapy product targeting tumour neoantigens. It is applicable to **any cancer type** for which neoantigens can be identified.

pTTL therapy is **personalised**, tailored for the patient's own tumour and taking advantage of the patient's immune cells.

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

A First-in Human trial of pTTL in stage IV **colorectal cancer** has been initiated.

pTTL production

- The bioinformatic software **PIOR® Manufacturing** is used for the identification, ranking and design of neoantigens for the manufacturing of pTTL.

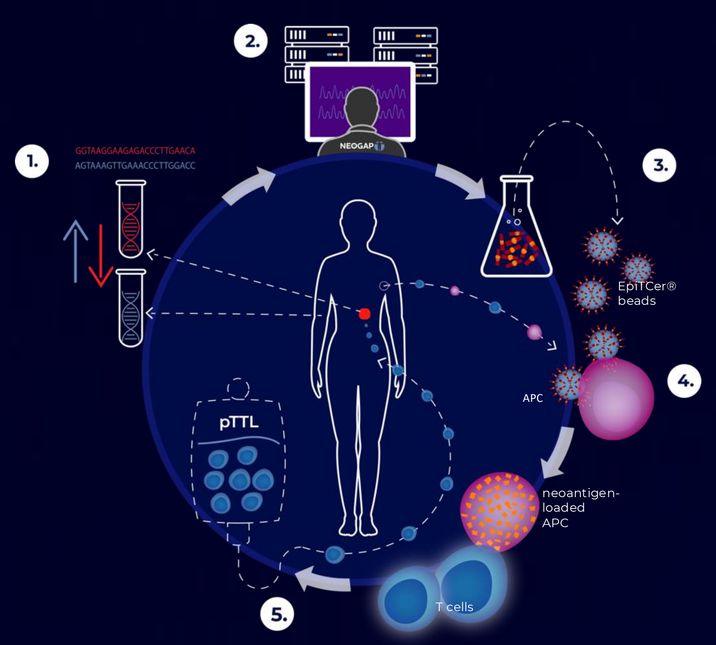
- The **EpiTCer® technology** offers a novel approach for efficient antigen delivery and specific T cell activation.

- **Starting material** for pTTL manufacturing is RLNs, which harbour an enriched population of tumour-experienced T cells[1-5].

- pTTLs are produced in a **controlled and reproducible GMP manufacturing process.**

1. *Marits et al 2006 Br J Cancer 94 1478-1484*
2. *Kim et al 1995 Cancer Biother 10:115–23*
3. *Triozzi et al 1995 J Natl Cancer 87: 1180 –1*
4. *Kim et al 1999 Cancer 86: 22-30*
5. *Zhen et al. 2015 Cancer Immunology, Imunotherapy 64:1083–1093*
6. *Sherif et al, 2010 Eur Urology 58: 105–111*

pTTL – 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.
4 Surgical collection of RLNs and *in vitro* culture with EpiTCer® beads in GMP compliant T cell expansion.
5. pTTL formulation and infusion to the patient.

Clinical study design

Phase I/IIa First-in-Human Trial

NCT05908643

Study design

Dose escalation with four groups receving an increasing dose

Part 1: Screening & pTTL manufacturing
Part 2: Preconditioning, treatment & 6 months follow up
Part 3: Long term follow up

Patients

Up to 16 (goal 12 evaluable) adult patients with colorectal cancer Stage IV :
- that 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
- that have measurable disease (RECIST1.1)
- present with a minimum life expectancy of 3 months at treatment

Study endpoints

Primary endpoint: safety

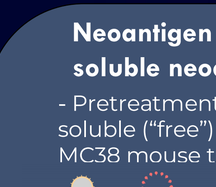
Secondary endpoints: standard clinical measurements:
- Objective response (iRESIST)
- time to and duration of response
- OS, PFS, disease-specific survival
- time to progression

Exploratory endpoints:
- Biomarker analyses (e.g. TCRseq, ctDNA)
- pTTL *in vivo* characterisation

Neoantigen selection by PIOR®

pTTL tumour targeting by PIOR® Manufacturing

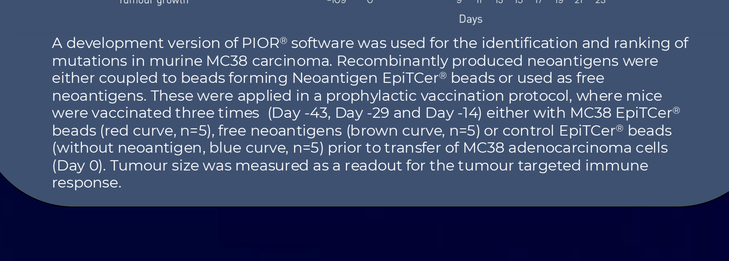
neoantigen identification, ranking and neoantigen construct design



PIOR® Manufacturing is a software for identification, selection and ranking developed by NEOGAP Therapeutics. Neoantigen constructs for EpiTCer® beads is also performed by PIOR® Manufacturing. Six neoantigen epitopes is included per construct and EpiTCer® beads with 3-6 neoantigen constructs are used for pTTL manufacturing.

EpiTCer® technology

Neoantigen selective T cell expansion is achieved with the EpiTCer® technology



Light microscope photo

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 GMP production and enables efficient removal of the beads from the cell therapy product. EpiTCer® technology promotes efficient antigen delivery, superior to soluble antigen delivery.

Neoantigen delivery by EpiTCer® beads compared to soluble neoantigens

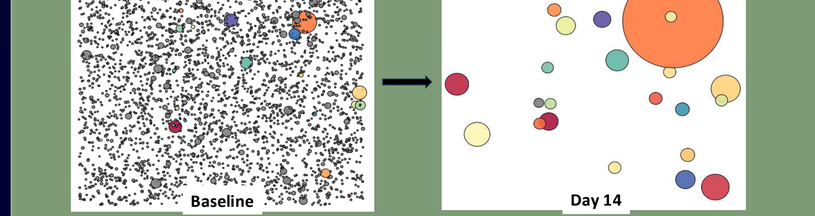
- Pretreatment with neoantigen EpiTCer beads, but not soluble ("free") neoantigen, delays tumour growth in the MC38 mouse tumour model

A development version of PIOR® software was used for the identification and ranking of mutations in murine MC38 carcinoma. Recombinantly produced neoantigens were either coupled to beads forming Neoantigen EpiTCer® beads or used as free neoantigens. These were applied in a prophylactic vaccination protocol, where mice were vaccinated three times (Day -43, Day -29 and Day -14) either with MC38 EpiTCer® beads (red curve, n=5), free neoantigens (brown curve, n=5) or control EpiTCer® beads (without neoantigen, blue curve, n=5) prior to transfer of MC38 adenocarcinoma cells (Day 0). Tumour size was measured as a readout for the tumour targeted immune response.

pTTL characterisation

T cell phenotype

- 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.



Phenotypic analysis by flow cytometry of 12 pTTL R&D batches.
A: Proportion of $CD4^+$, $CD8^+$ and double negative ($CD3^+CD4^-CD8^-$; DN) T cells among $CD3^+$ T-cells.
B: Proportion of $CD3^+$ T cells expressing activation markers PD1, CTLA4 and both PD1 and CTLA4.
C: Proportion of $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells expressing costimulatory receptor CD28.
D: Differentiation state by proportion of central/effector memory T cells, Tcm+Tem $CD45RA^-CCR7^{+/-}$, and fully differentiated T cells, Temra $CD45RA^+CCR7^-$, among $CD3^+CD4^+$ and $CD3^+CD8^+$ T cells.

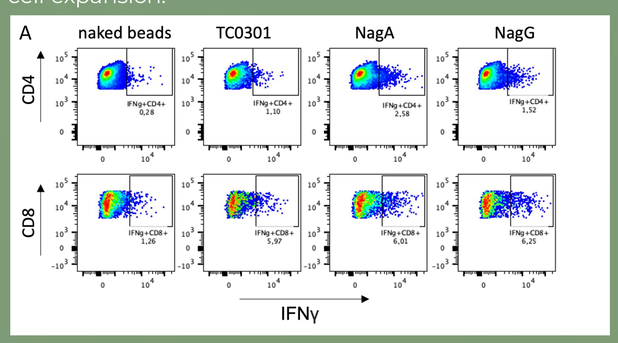
T cell clonality

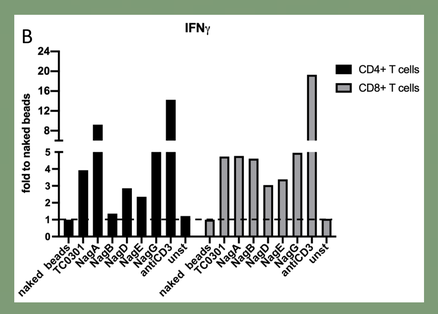
- The pTTL production process generates an **oligoclonal T cell product**.

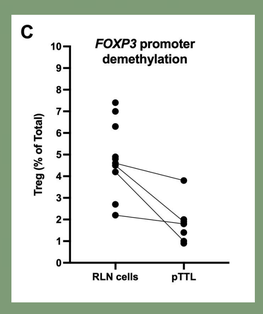
Clonal expansion of T cells during pTTL production analysed by TCRalpha and TCRbeta sequences at baseline and in corresponding pTTL products.
A: Sum proportion of the 15 largest clones at baseline, i.e. in RLN starting material, and for corresponding pTTL products are shown for TCRα and TCRβ, based on sequences of the CDR1, CDR2 and CDR3 sections of the TCR chains. Batch #27 and #28 originates from the same starting material.
B: Graphical representation of T cell clones at baseline and in pTTL (Day 14) for the #24 pTTL product. Each circle represents a unique TCR sequence. Identity of clones is represented by color and abundance by circle size.

T cell functionality

- pTTL shows neoantigen specific activation towards the personalised EpiTCer® beads used for neoantigen selective T cell expansion during pTTL production.
- EpiTCer® bead stimulation does not favour regulatory T cell expansion.

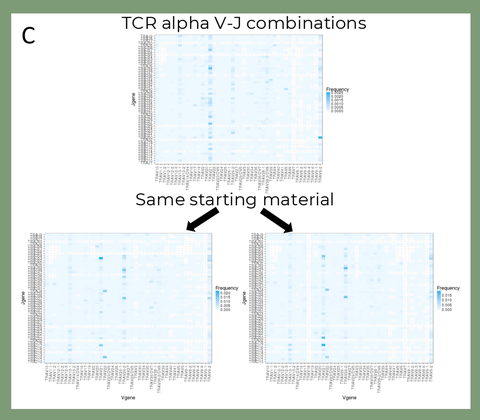






A: Intracellular INFgamma analysis by flow cytometry of a pTTL product *in vitro* restimulated with "naked beads" (neg control), TC0301 (mix of 5 EpiTCer® beads) and two separate EpiTCer® beads, NagA and NagG. Gated on CD4+ and $CD8^+$ $CD3^+$ T cells.
B: Fold increase of INFgamma positive CD4 and CD8 T cells compared to neg control (naked beads) after *in vitro* restimulation with TC0301 (mix of 5 EpiTCer® beads) and the 5 separate EpiTCer® beads, NagA, NagB, NagD, NagE and NagG, included in TC0301. Positive control=antiCD3 stimulation, unst= medium only.
C: Proportion of bona fide regulatory T cells before (RLN cells) and after culture (pTTL) by analysis of *FOXP3* promoter demethylation (PureQuant™ Treg assay).

- Overlapping T cell clonality from repeated pTTL productions of the same starting material supports specificity towards EpiTCer® presented neoantigens



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.
D: TRA V-J combination heatmaps of RLN cells (starting material from CRC patient, upper panel) and two pTTL batches from the same starting material (lower panels).

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