Poster 437

Personalized Tumor-Trained Lymphocytes – A neoantigen targeted T cell therapy product for treatment of colorectal cancer

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Introduction

pTTL (personalized tumor-Trained Lymphocytes) is an adoptive T cell therapy product targeting tumor neoantigens. It is applicable to **any cancer type** for which neoantigens can be identified.

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

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

A First-in Human trial of pTTL is conducted in in stage IV **colorectal cancer** patients.

pTTL production

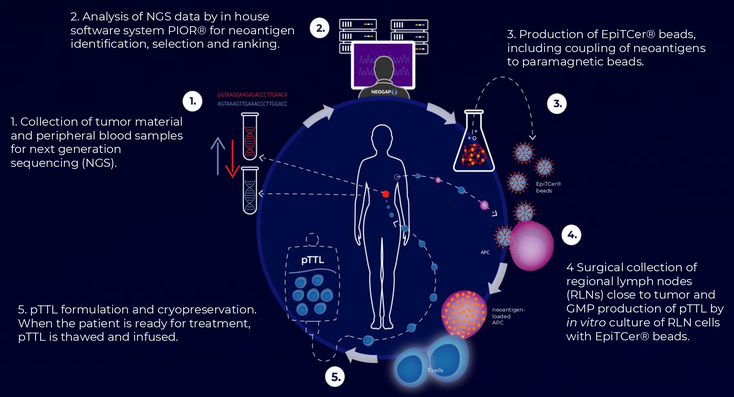
- The bioinformatic software **PIOR® Manufacturing** is used for the identification, ranking and design of neoantigens for the manufacturing of pTTL.

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

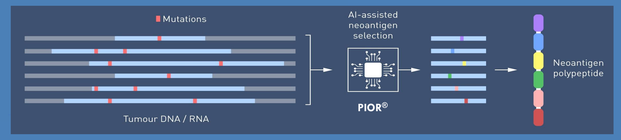
- **Starting material** for pTTL manufacturing is RLNs, which harbour an enriched population of tumor-experienced T cells[1-6].

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

pTTL – overview



Neoantigen identification, ranking and design by PIOR®

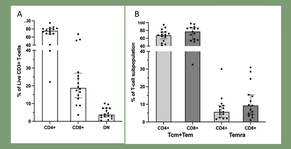


PIOR® is a software for neoantigen identification, selection and ranking developed by NEOGAP Therapeutics. Neoantigen constructs for EpiTCer® beads are designed by PIOR®. Six neoantigen epitopes is included per construct and EpiTCer® beads with 3-6 neoantigen constructs are used for pTTL manufacturing.

pTTL characterization

pTTL phenotype

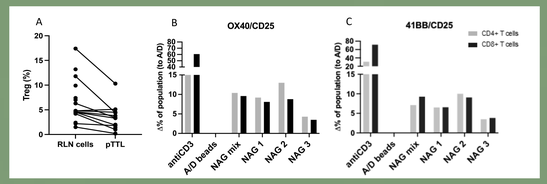
- Individual variation in proportion of $CD4^+$ / $CD8^+$ T cells.
- Mainly composed of Tcm or Tem cells.



Phenotypic analysis by flow cytometry (n=16 pTTL batches).
A: Proportion of $CD4^+$, $CD8^+$ and double negative ($CD3^+CD4^-CD8^-$; DN) T cells among $CD3^+$ T-cells.
B: Differentiation state by proportion of central/effector memory T cells, Tcm+Tem $CD45RA^-CCR7^{+/-}$, and fully differentiated T cells, Temra $CD45RA^+CCR7^-$, among $CD3^+CD4^+$ (light grey), and $CD3^+CD8^+$ (dark grey) T cells.

pTTL functionality

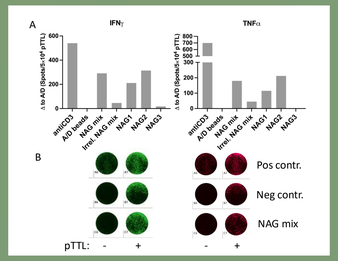
- Neoantigen specific activation in pTTL in response to restimulation with the personalized EpiTCer® beads used for pTTL production.
- pTTL manufacturing process does not favour regulatory T cell expansion.



A: Proportion of regulatory T cells (Treg) in starting material (RLN cells) and cell product (pTTL) by analysis of *FOXP3* promoter demethylation (PureQuant™ Treg assay). N=13 paired RLN/pTTL data.
B and C: Expression of activation induced marker OX40 (B) and 4-1BB (C) on $CD25^+CD4^+$ (light grey) and $CD25^+CD8^+$ (dark grey) T cells in response to recall stimulation with a pool of 3-6 personalized EpiTCer® beads (same as used for pTTL production, NAG mix) or separate EpiTCer® beads (NAG 1-3, included in NAG mix). Positive control: anti-CD3; negative control: activated/deactivated (A/D) beads. Example from one patient shown (the FluoroSpot data shown in a separate figure were generated from the same patient). Flow cytometry data expressed as "Δ% of population (to A/D)", i.e. % expression of activation induced markers in relation to marker expression on A/D beads.

pTTL functionality

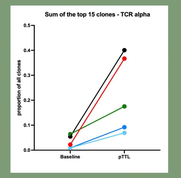
- Neoantigen specific cytokine production towards the personalized EpiTCer® beads used for pTTL production.



A. Quantification by FluoroSpot of IFNγ, and TNFα positive T cells upon *in vitro* restimulation with a pool of personalized EpiTCer® beads (the same as used for pTTL production, NAG mix), pool of irrelevant EpiTCer beads (irrel. NAG mix, personalized for another patient) or separate EpiTCer® beads (NAG 1-3, included in NAG mix). Positive control: anti CD3, negative control activated /deactivated (A/D) beads.
B. Corresponding FluoroSpot assay images.
IFNγ, TNFα values are normalized to the A/D beads condition. Example from one patient shown.

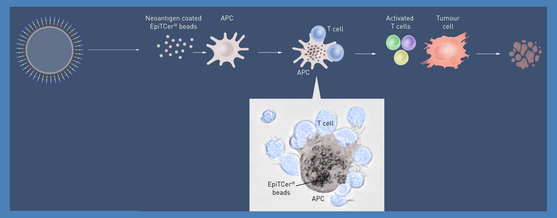
T cell clonality

- Oligoclonal T cell product.



Clonal expansion of T cells during pTTL production analysed by TCRα and TCRβ sequences (data shown for TCRα) at baseline and in pTTL products. Sum proportion of the 15 largest clones in RLN starting material (baseline) and corresponding pTTL product, based on sequences of the CDR1, CDR2 and CDR3 sections of the TCR chains. The dark and light blue symbols represent batches originating from the same starting material.

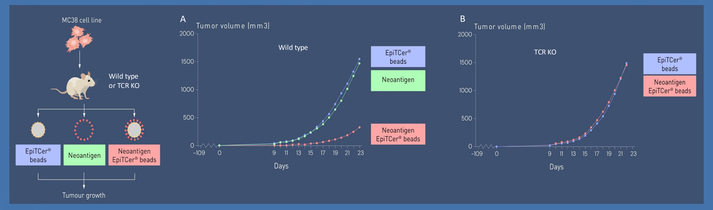
Neoantigen selective T cell expansion using EpiTCer® technology



EpiTCer® beads, 1 µm size, promote efficient phagocytosis, neoantigen processing and presentation by antigen presenting cells (APCs). The process employs natural antigen processing, is **HLA agnostic** and **promotes cross-presentation**, activating both $CD4^+$ and $CD8^+$ T cells. The beads are paramagnetic, allowing sterilization and processing compatible with GMP production, and enabling removal of the beads from the cell product.

EpiTCer® technology promotes efficient antigen delivery

- MC38 tumor model
- Superior tumor growth inhibition by Neoantigen EpiTCer® beads compared to free neoantigens
- Tumor growth inhibition is mediated by T cells



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, "Neoantigen EpiTCer® beads", or used as free neoantigens. These were applied in a prophylactic vaccination protocol, where wt mice (A) or TCR KO mice (B) were vaccinated three times (Day -43, Day -29 and Day -14) either with Neoantigen EpiTCer® beads (red curve, n=5), free neoantigens (green curve, n=5) or control EpiTCer® beads (without neoantigen, blue curve, n=5) prior to transfer of MC38 adenocarcinoma cells (Day 0). Tumor size (mm^3) was measured as readout for tumor targeted immune response.

Phase I/IIa First-in-Human Trial ongoing

Trial design

Dose escalation with four groups receiving an increasing cell dose.

Part 1: Screening & pTTL manufacturing
Part 2: Preconditioning (3x cyclophosphamide, 300 mg/m^2 BSA + fludarabin, 30 mg/m^2 BSA), pTTL treatment, 6 months follow-up
Part 3: Long term follow-up

Patients

Up to 16 adult patients with Stage IV colorectal cancer

Trial endpoints

Primary endpoint: safety and tolerability

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* characterization

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References
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