# Potency Assay Development for the characterization of personalized Tumour-Trained Lymphocytes

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## Background

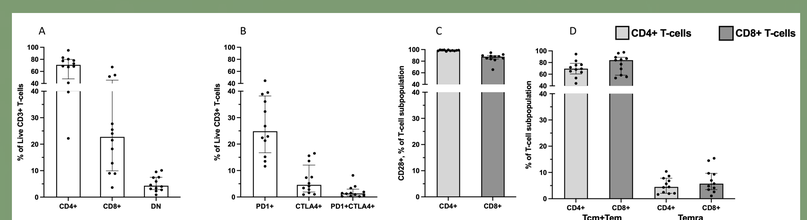
Potency assays are critical for the quality control of personalized T cell products and ideally will detect useful biomarkers of efficacy *in vivo.*

Here we describe an assay development method for **pTTL** (**p**ersonalized **T**umour-**T**rained **L**ymphocytes). pTTL originate from autologous regional lymph nodes (RLN) and is applicable to any cancer type for which neoantigens can be identified. Selective expansion of neoantigen specific T cells is achieved by *in vitro* stimulation with EpiTCer® beads; paramagnetic beads conjugated to neoantigen epitopes identified from sequence data from the patient's tumour.

For the development of potency assays, here we used **surrogate pTTL (spTTL)** made from Plasma Blood Mononuclear Cells (PBMCs) of healthy pp65-responding individuals and EpiTCer® beads conjugated to cytomegalovirus pp65 antigen. Parameters selected may have a clinical application for an adoptive T cell therapy against colorectal cancer.

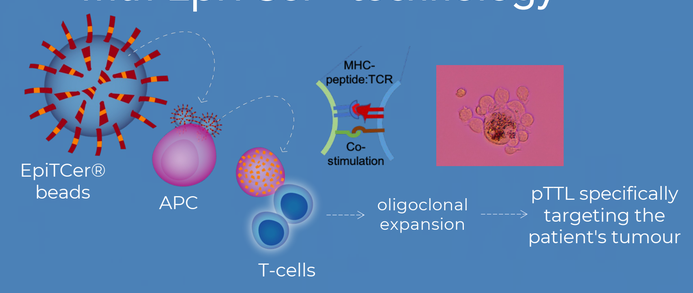
## pTTL characterization/T cell phenotype

- 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.



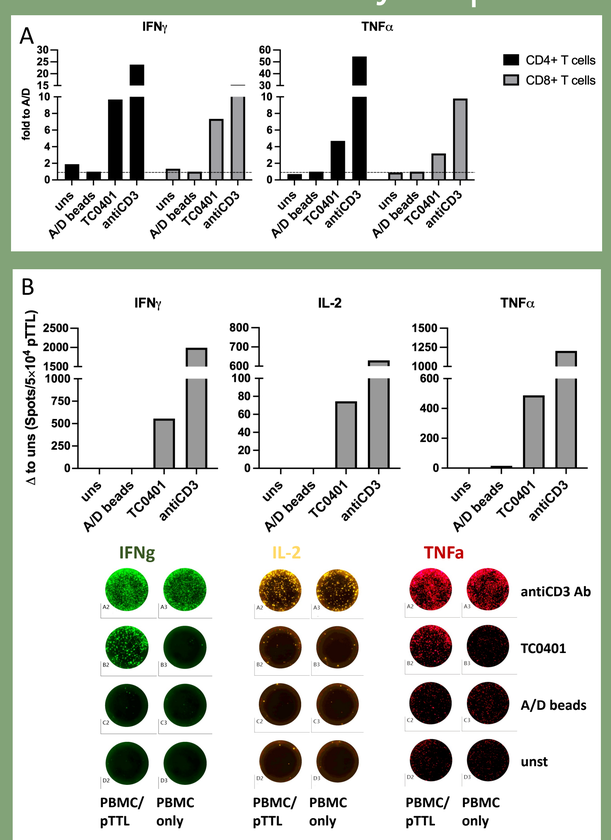

Phenotypic analysis by flow cytometry of 12 pTTL R&D batches.
A: Proportion of $CD4^+$, $CD8^+$ and double negative ($CD3^+CD4^-CD8^-$; DN) T cells among $CD3^+$ T-cells.
B: Proportion of $CD3^+$ T cells expressing activation markers PD1, CTLA4 and both PD1 and CTLA4.
C: Proportion of $CD3^+CD4^+$ and $CD3^+CD8^+$ 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.

## Neoantigen selective T cell expansion with EpiTCer® technology




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.

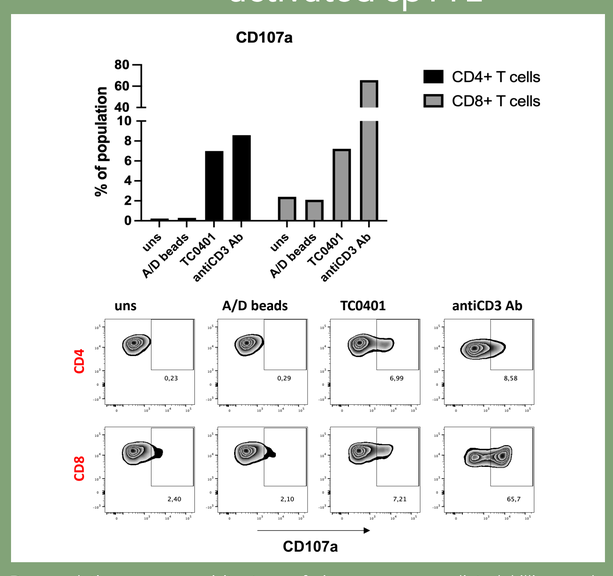
## Upregulation of cytokines with anti-tumor activity in spTTL




A: Fold increase of cytokine-positive CD4+ and CD8+ T cells as compared to neg control (A/D beads) assessed by intracellular cytokine staining and flow cytometry after *in vitro* restimulation with TC0401.
B: FluoroSpot analyses of spTTL in response to recall antigen stimulation. Quantification of cytokine-positive cells (upper panel) and the corresponding FluoroSpot assay images (lower panel) are shown. Cytokine values are normalized to the A/D beads condition.

**(uns)** medium only, **(A/D beads)** EpiTCer® beads without any antigen which have been chemically activated and deactivated during amino coupling procedure, **(TC0401)** EpiTCer® beads conjugated to cytomegalovirus pp65 peptides as surrogate antigen, **(antiCD3)** positive control.

## Increased surface expression of CD107a on activated spTTL




Degranulation, a prerequisite to perforin-granzyme–mediated killing and required for immediate lytic function mediated by responding antigen-specific T cells, was evaluated by flow cytometric analysis of the lysosomal-associated membrane glycoprotein 1 (LAMP-1 or CD107a) on antigen stimulated CD4+ and CD8+ T cells within spTTL. Quantification (upper panel) and corresponding FACS plots (lower panel) are shown.

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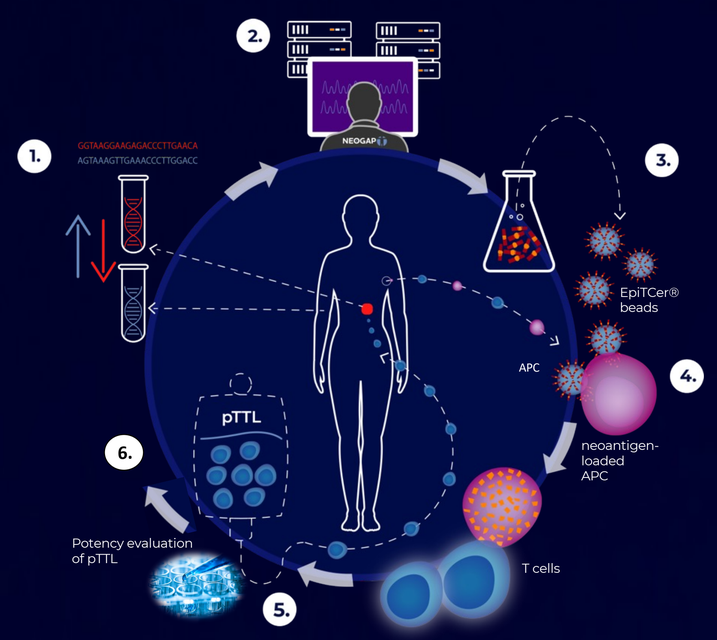
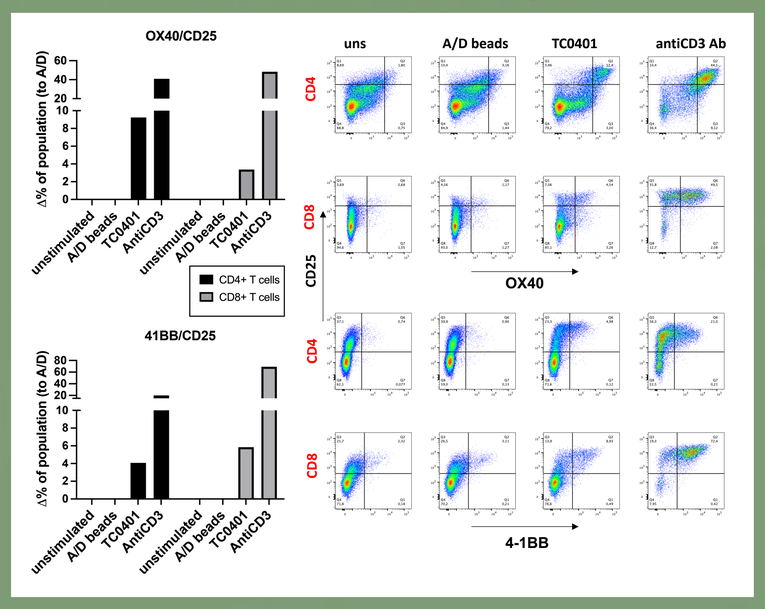
## pTTL Overview




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. *in vitro* potency evaluation of pTTL.**
6. pTTL formulation and infusion to the patient.

## Upregulation of T cell activation-induced markers on spTTL




Functionally diverse antigen-specific T cells were analysed by flow cytometry, measuring upregulation of activation markers, 4-1BB/CD25 and OX40/CD25 upon *in vitro* antigen stimulation. Quantification (left) and representative gating (right) are shown.

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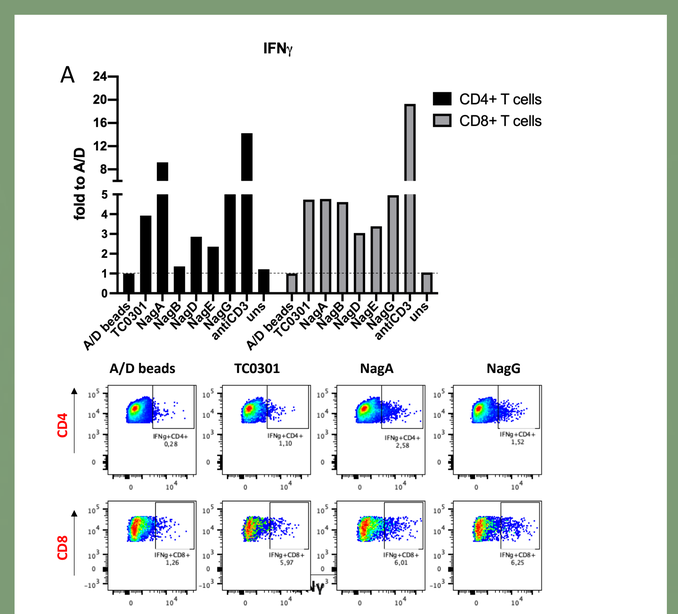
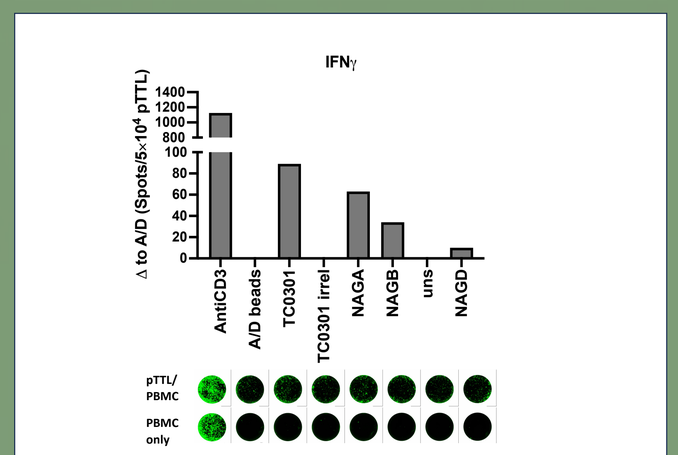
## pTTL functionality

pTTL shows neoantigen-specific activation by the personalized EpiTCer® beads used for selective T cell expansion during pTTL production.




A: Fold increase of IFNγ-positive CD4+ and CD8 T cells compared to neg control (A/D beads) after *in vitro* restimulation with TC0301 and the 5 separate EpiTCer® beads, neoantigen (Nag)A, NagB, NagD, NagE and NagG (upper panel) and representative flow cytometry plots (lower panel).

B: Quantification of IFNγ-positive T cells upon *in vitro* restimulation with neoantigens by FluoroSpot (upper panel) and corresponding FluoroSpot assay images (lower panel). IFNγ values are normalized to the A/D beads condition.

**(uns)** medium only, **(A/D beads)** EpiTCer® beads without neoantigen which have been chemically activated and deactivated during amino coupling procedure, **(TC0301)** mix of 5 EpiTCer® beads conjugated to a CRC patient's tumour neoantigens that are used for assessing functionality of pTTL generated from the same patient, **(TC0301 irrel)** irrelevant mix of 5 EpiTCer® beads conjugated to neoantigens identified in and produced for another patient's tumour, **(antiCD3)** positive control.

## Conclusion

Parameters selected using spTTL have the potential to be applied for the characterisation of pTTL and may be incorporated as potency assays for clinical application.

## Current Trial Information

- Clinical Trial Number: Eudra CT 2022-000394-96
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