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

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Background

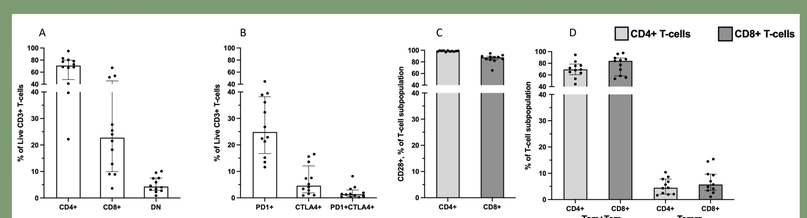
Potency assays are critical for the quality control of personalized T cell products and ideally will detect useful biomarkers of efficacy *in vivo.*

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.

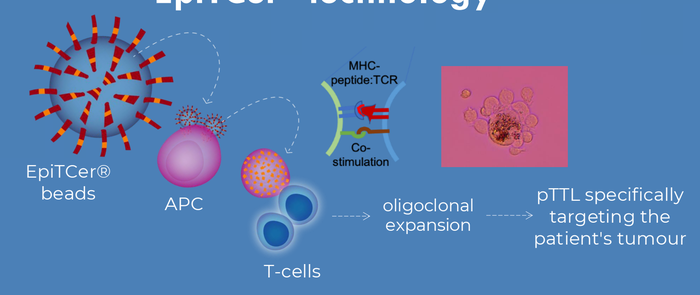
pTTL characterization/T cell phenotype

- 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.



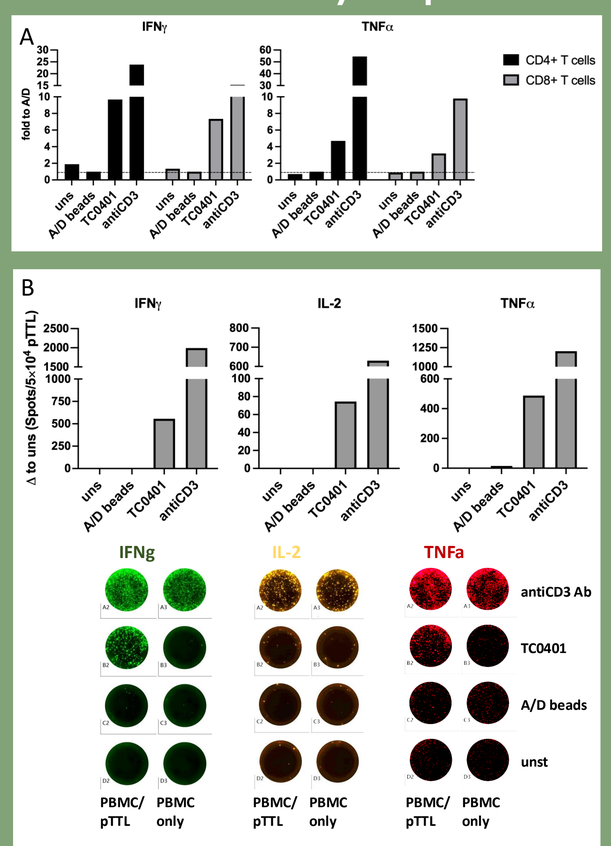
Phenotypic analysis by flow cytometry of 12 pTTL R&D batches.
A: Proportion of $CD4^+$, $CD8^+$ and double negative ($CD3^+CD4^-CD8^-$; DN) T cells among $CD3^+$ T-cells.
B: Proportion of $CD3^+$ T cells expressing activation markers PD1, CTLA4 and both PD1 and CTLA4.
C: Proportion of $CD3^+CD4^+$ and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

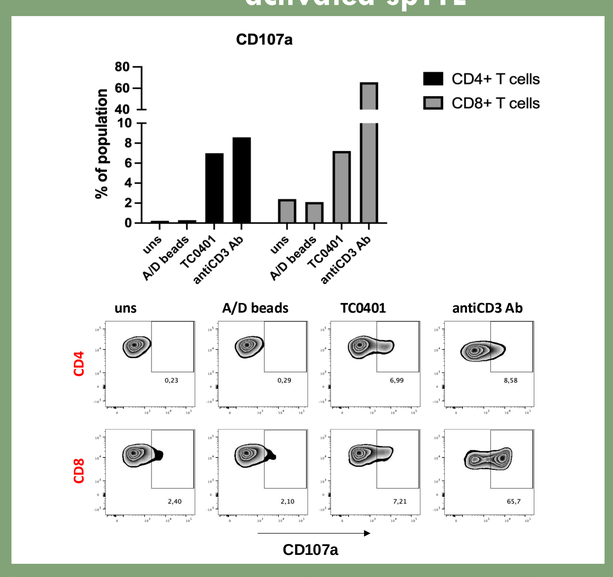
Upregulation of cytokines with anti-tumor activity in spTTL



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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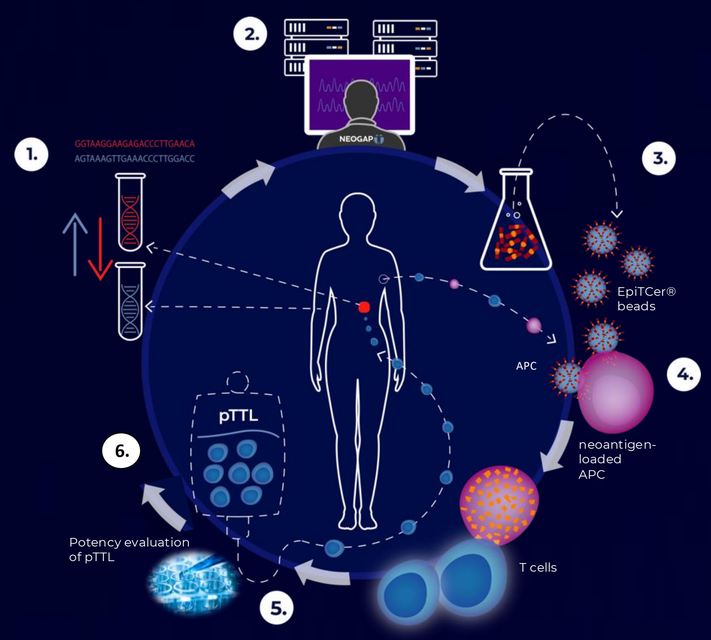
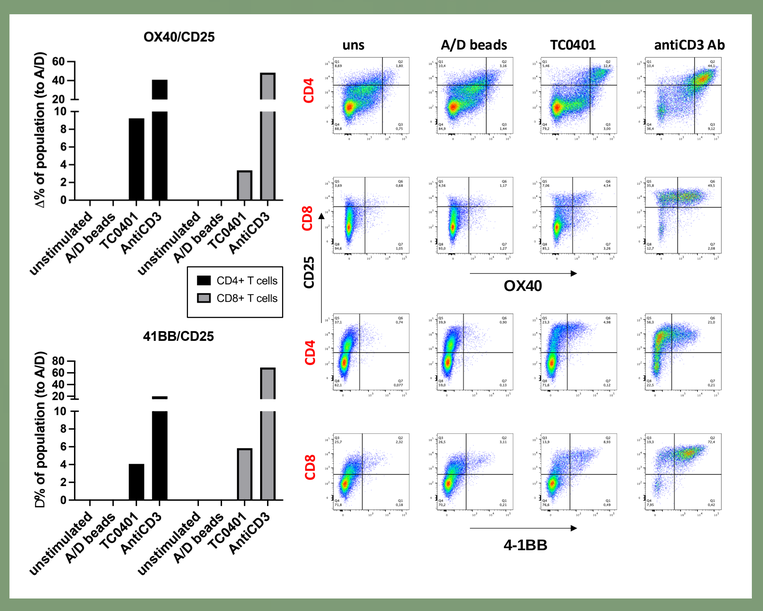
pTTL Overview



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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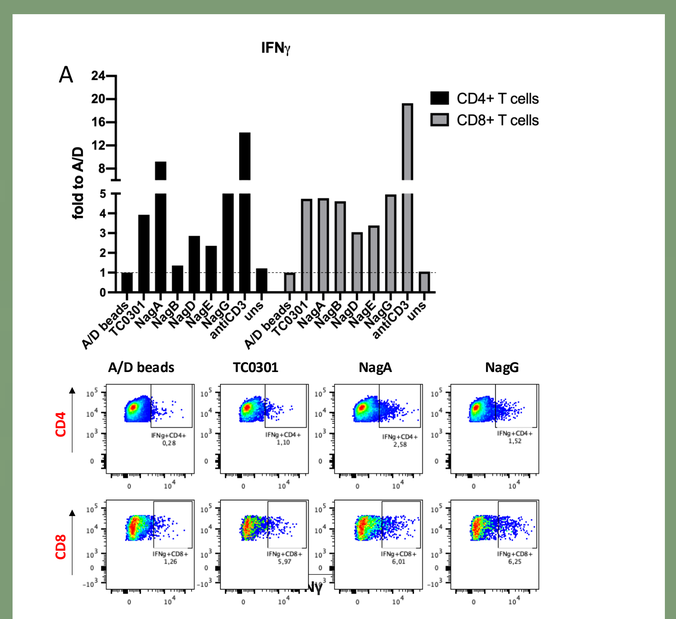
pTTL functionality

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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