Immunological activity of oncolytic adenovirus encoding TNFα and IL-2 (TILT-123) in combination with pembrolizumab in platinum resistant or refractory ovarian cancer patients

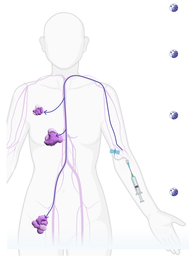
Abstract# **CT139**

J. H. A. Clubb[1], M. S. Block[2], J. Mäenpää[3], S. Pakola[4], L. Haybout[1], D. C. A. Quixabeira[1], T. Kudling[4], M. van der Heijden[4], E. Jirovec[1], T. Alanko[3], D. A. Adamo[5], S. Ramadan[3], J. Sormunen[3], J. Kononen[3], J. W. Cohen[6], N. Singh[6], J. Goldfinch[1], S. Sorsa[1], R. Havunen[1], C. Kistler[1], J. M. Santos[1], V. Cervera-Carrascon[1], A. Hemminki[1]

1)TILT Biotherapeutics, Helsinki, Finland, 2)Mayo Clinic Cancer Center, Rochester, MN, 3)Docrates Cancer Center, Helsinki, Finland, 4)University of Helsinki, Helsinki, Finland, 5)Mayo Clinic, Rochester, MN, 6)Merck & Co., Inc., Rahway, NJ USA

This study is in collaboration with Merck Sharp & Dohme LLC, a subsidiary of Merck & Co., Inc., Rahway, NJ, USA

1. Background



- Immune checkpoint inhibitors have so far failed to benefit most patients with ovarian cancer;
- Novel approaches to sensitize the ovarian tumour immune microenvironment to immune checkpoint blockade are needed to improve clinical outcomes;
- TILT-123, a serotype chimeric oncolytic adenovirus encoding tumor necrosis factor alpha (TNFa) and Interleukin-2 (IL-2), designed to improve T cell therapies, was evaluated with anti-PD-1 pembrolizumab.
- Analysis of samples collected during the trial enables evaluation of mechanism of action and identification of biomarkers.

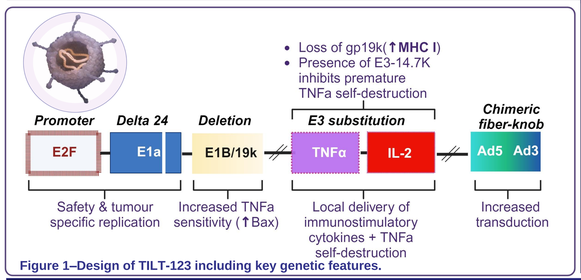


Figure 1–Design of TILT-123 including key genetic features.

2. Trial Design & Sampling

T-563 - PROTA (NCT05271318)

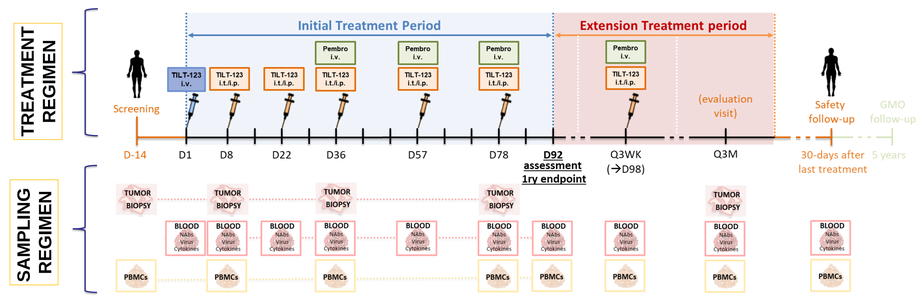


Figure 2– Clinical trial design and sample collection schedule

- Open label phase I clinical trial using a standard 3+3 dose escalation scheme.
- Platinum refractory or platinum resistant ovarian cancer with at least one intratumorally or intraperitoneally injectable tumor.
- Four tumor biopsies, seven serum samples and five PBMC samples collected for evaluation of secondary and exploratory endpoints.

Primary endpoint	Secondary endpoints		Exploratory Endpoints
Safety by day 92	Maximum tolerated dose (MTD).	Immune response towards virus and tumor.	Evaluation of biological changes in tumors or metastases.
	Tumor response (iRECIST and RECIST 1.1), PFS and OS.	Virus persistence.	Biomarker discovery
	Changes in CA125.	Virus shedding.	

	Virus i.v. /dose (VP)	Virus i.t./i.p. /dose (VP)	pembrolizumab i.v/dose (mg)
Cohort 1	$3x10^{11}$	$1x10^{11}$	200
Cohort 2	$1x10^{12}$	$3x10^{11}$	200
Cohort 3	$2x10^{12}$	$3x10^{11}$	200
Cohort 4	$4x10^{12}$	$5x10^{11}$	200

Figure 3– Dosing strategy for cohorts 1 – 4.

- Patients receive an intravenous dose of TILT-123 on day 1 ranging from $3x10^{11}$ to $4x10^{12}$ VP, delivered in a volume ranging from 10-40ml.
- Patients receive intratumoral or intraperitoneal doses of TILT-123 on day 8, 22, 36, 57 and 78 ranging from $1x10^{11}$ to $5x10^{11}$ VP, delivered as a total volume of 4ml for intratumoral and 50ml for intraperitoneal injections.
- Patients receive 200mg intravenous doses of pembrolizumab on day 36, 57 and 78.

3. Demographics & response

Patient ID	Cohort	Age	ECOG status at baseline	Time since diagnosis (months)	Cancer type	Histological Subtype	Number of previous systemic treatments	Platinum status	ICI status	Best Overall response RECIST 1.1	Best Overall response iRECIST	Best CA125 change, d = day	OS (days)
302-05	1	60	1	63	Fallopian tube cancer	HGSOC	7	Resistant	Naive	PD	iUPD	-8.1%, d-14 vs d8	85
302-06	1	70	1	282	Epithelial ovarian cancer	LGSOC	12	Resistant	Naive	SD	iSD	-37.5%, d-14 vs d36	306
301-01	1	71	1	30	Primary peritoneal cancer	Serous	4	Resistant	Naive	SD	iSD	+348.3%, d-14 vs d36	77
301-02	2	61	1	13	Epithelial ovarian cancer	HGSOC	3	Resistant	Naive	SD	iSD	-17.8%, d36 vs d78	434
301-03	2	72	1	148	Epithelial ovarian cancer	Serous adenocarcinoma	12	Resistant	Naive	SD	iSD	+62.6%, d-14 vs d36	122
301-04	2	58	1	62	Epithelial ovarian cancer	Serous	7	Resistant	Refractory	PD	iUPD	+24.9%, d-14 vs d36	93
302-07	3	66	1	26	Fallopian tube cancer	HGSOC	5	Refractory	Naive	SD	iSD	-15.1%, d49 vs d57	190
301-05	3	78	0	15	Epithelial ovarian cancer	HGSOC	1	Resistant	Naive	SD	iSD	-35.1%, d57 vs d100	369*
302-09	3	51	1	80	Epithelial ovarian cancer	HGSOC	8	Refractory	Naive	PD	iUPD	-14.8%, d-14 vs d57	280
302-10	4	54	0	59	Epithelial ovarian cancer	HGSOC	11	Refractory	Naive	SD	iSD	-28.1%, d-14 vs d78	160*
301-10	4	58	0	48	Fallopian tube cancer	HGSOC	5	Resistant	Naive	PD	iUPD	+56.1%, d-14 vs d8	151*
302-11	4	36	0	45	Primary peritoneal cancer	Adenocarcinoma LGSOC	9	Refractory	Naive	N/A	N/A	+26.1%, d-14 vs d8	149*
301-11	4	69	0	36	Epithelial ovarian cancer	Mucinous carcinoma	4	Refractory	Naive	PR	iPR	-7.1%, d-14 vs d57	130*
301-12	4	73	0	20	Epithelial ovarian cancer	Carcinosarcoma	3	Refractory	Naive	SD	iSD	+3.8%, d-14 vs d8	123*
302-12	4	71	0	76	Primary peritoneal cancer	HGSOC	8	Resistant	Naive	SD	iUPD	-34%, d-14 vs d36	97*

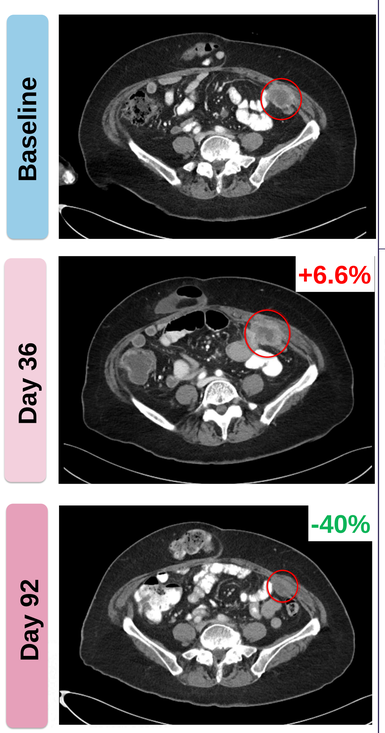
Table 1– Demographics of patients enrolled in cohorts 1 – 4.* Patient still alive (18 Mar 2024)

- A total of 15 patients were enrolled onto the trial with a median age of 66 years.
- Prevalence of cancer types were epithelial ovarian cancer (60%), fallopian tube cancer (20%) and primary peritoneal cancer (20%).
- Percentage of platinum refractory and resistant cancers were 47% and 53% respectively.

4. Anti-tumor activity & virotherapy induced immunomodulation

T-563 - PROTA (NCT05271318)

Example 1 – CT images of injected abdominal wall metastasis from patient 301-11 with mucinous ovarian cancer.



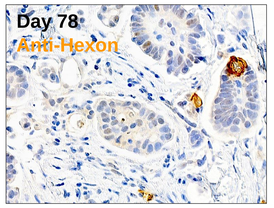


Figure 4 – Immunohistochemistry of anti-hexon stain on day 78 of a non-injected tumor from patient 302-06.

- Adenovirus protein was detected in injected and non-injected tumors including the tumor biopsy above taken 21 days after the previous treatment. These findings suggest TILT-123 is able to replicate and systemically disseminate.

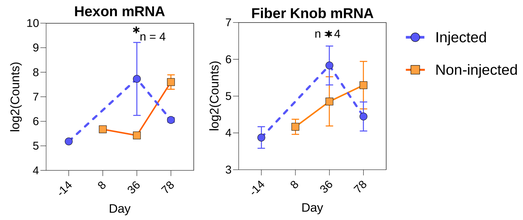


Figure 5 – Quantification of adenovirus mRNA in injected and non-injected tumors

- TILT-123 was detected by analysis of adenovirus mRNA in tumor biopsies. A significant increase in hexon and fiber knob mRNA was detected in injected lesions at day 36 when compared to baseline. Interestingly, the value dropped by day 78 but increased in non-injected tumors supporting the notion of dissemination of virus from injected to non-injected tumors.

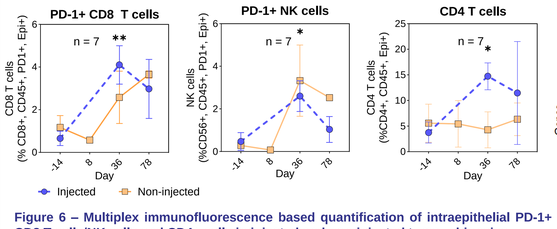


Figure 6 – Multiplex immunofluorescence based quantification of intraepithelial PD-1+ CD8 T cells/NK cells and CD4+ cells in injected and non-injected tumour biopsies.

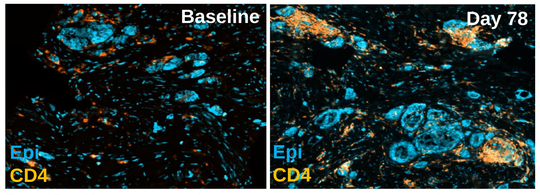


Figure 7 – Multiplex immunofluorescence showing an increase of mature tertiary lymphoid structures, in an injected lesion from long term survivor 302-06. Percentage of CD4+ clusters in orange were also positive for CD20, CD11c, PD-L1 and Foxp3.

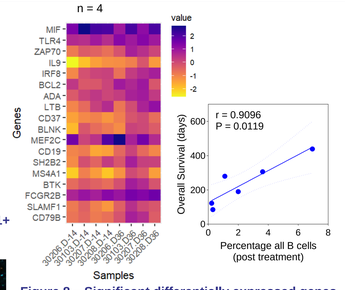


Figure 8 – Significant differentially expressed genes in injected tumors based on four patients. Gene panel displayed focuses on B cell related genes. Significant correlation of percentage of CD20+ B cells post treatment in injected tumors and overall survival.

- Analysis of tumor biopsies revealed significant treatment related immunomodulation including increase percentage of PD-1 expressing cytotoxic immune cells as well as induction of tertiary lymphoid formation.
- Activation of the B cell compartment, known to be a key player in ovarian cancer immunity, was also observed.

5. Neutralizing antibodies & systemic humoral response

T-563 - PROTA (NCT05271318)

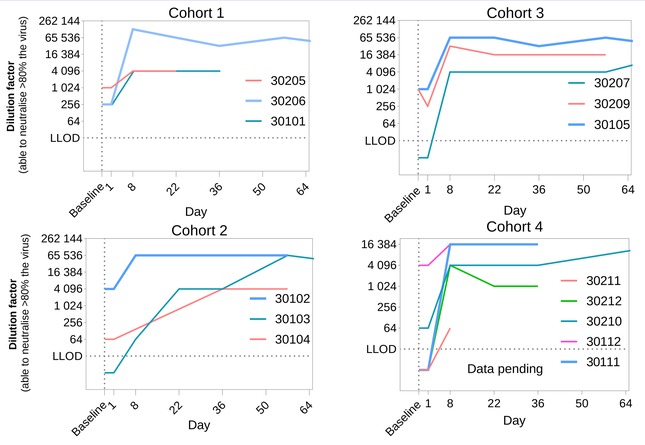


Figure 9– Analysis of neutralizing antibody titre against adenovirus present in serum during treatment.

- Neutralizing antibodies against adenovirus were detected in 77% of patients at baseline.
- Neutralizing antibodies increase in all patients but high variability in maximum titre achieved was observed.
- Maximum titre detected was 1:262144 at day 8 in patient 30206 who was amongst the longer term survivors.

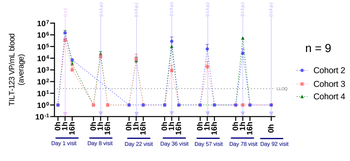


Figure 12– Quantification of circulating TILT-123 in whole blood as measured by qPCR.

- Samples were taken at 0 hour, 1 hour and 16 hour on each sampling day.
- Maximum virus concentration detected in blood was 1 hour after i.v. delivery at 10^6 VP/ml. Virus was also detected in blood after intratumoral delivery.

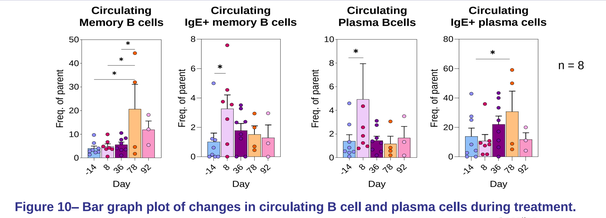


Figure 10– Bar graph plot of changes in circulating B cell and plasma cells during treatment.

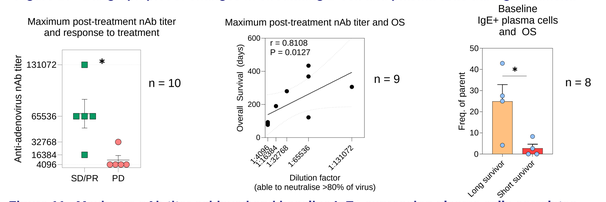


Figure 11– Maximum nAb titer achieved and baseline IgE+ expressing plasma cells correlates with response and overall survival.

- Significant increase in circulating memory B and plasma cells on day 78 and day 8 respectively.
- Higher nAb titer following treatment significantly correlates with response and survival suggesting critical role of humoral response in efficacy (more antibodies = longer survival).
- Higher frequency of baseline IgE+ plasma cells significantly correlates with overall survival.

6. Summary and conclusions

- Disease control was seen in 10/14 (71%) evaluable patients including one long lasting partial response in a patient with mucinous carcinoma
- Tumor size reductions were seen in injected and non-injected tumors.
- TILT-123 was detected in injected and non-injected tumors at the protein and mRNA level.
- Significant immunomodulation in injected and non-injected tumors was observed, by multiple immunofluorescence and gene expression analysis.
- Strength of the humoral response and presence of IgE+ plasma cells at baseline significantly correlated with response and overall survival.
- **Biomarker summary-** The identification and quantification of antibody-producing cells can be used as a preliminary measure to assess the efficacy of TILT-123. This encouraging data needs to be validated through larger studies.

For more information contact:

James Clubb
Proejct Manager at TILT Biotherapeutics
Email: james.clubb@tiltbio.com

We thank the patients and staff at:

DOCRATES CANCER CENTER, MAYO CLINIC, HUS Helsinki University Hospital

We thank the funding bodies including:

Ida Montinin Säätiö, Syöpäsäätiö Cancer Foundation Finland, Sigrid Jusélius Foundation, Cancer Society of Finland, K. Albin Johanssons Stiftelse, Minerva Foundation, DoD Study ID: OC220391, ORION

This project has received funding from the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No 813453.

Study sponsored by:

https://tiltbio.com/