#1518

T-cell inducing oncolytic virus (igrelimogene litadenorepvec; TILT-123) shows safety, anti-tumor activity and induction of immune responses in advanced solid tumor patients (full report on TUNIMO)

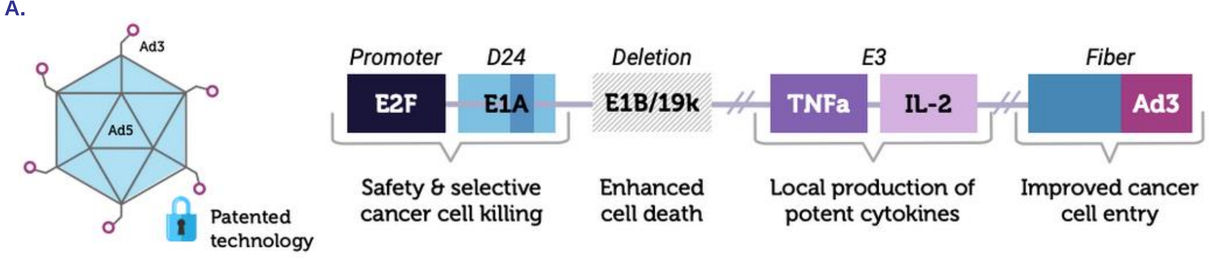
J.M Santos[1,2], K. Peltola[3], T. Alanko[4], S. Pakola[2], J. Clubb[1,2], E. Jirovec[2], L. Haybout[1,2], T. Kudling[2], R. Korpisaari[4], S. Juteau[5], M. Jaakkola[5], J. Sormunen[4], J. Kononen[4], D.C.A. Quixabeira[1,2], C. Kistler[1], S. Sorsa[1,2], R. Havunen[1,2], V. Cervera-Carrascon[1,2], A. Hemminki[1,2,3].

1) TILT Biotherapeutics Ltd, Helsinki, Finland, 2) Cancer Gene Therapy Group, Translational Immunology Research Program, University of Helsinki, Helsinki, Finland , 3) Comprehensive Cancer Centre, Helsinki University Hospital, Helsinki, Finland, 4) Docrates Cancer Center, Helsinki, Finland, 5) HUS Helsinki University Hospital

Background

- While delivering cancer therapy breakthroughs, immunotherapy is not able to provide benefit in most patients;
- In addition to tumor heterogeneity in patients, the tumor microenvironment is convoluted and infiltrating T-cells (or lack thereof) are a consistent target of suppression across different tumor types;
- **Igrelimogene litadenorepvec** (Ad5/3-E2F-d24-hTNF-IRES-hIL2; TILT-123), is an oncolytic adenovirus encoding for interleukin-2 and tumor necrosis factor alpha, designed for recruiting, propagating and stimulating T-cells for re-invigoration of the tumor microenvironment.

A.



B.

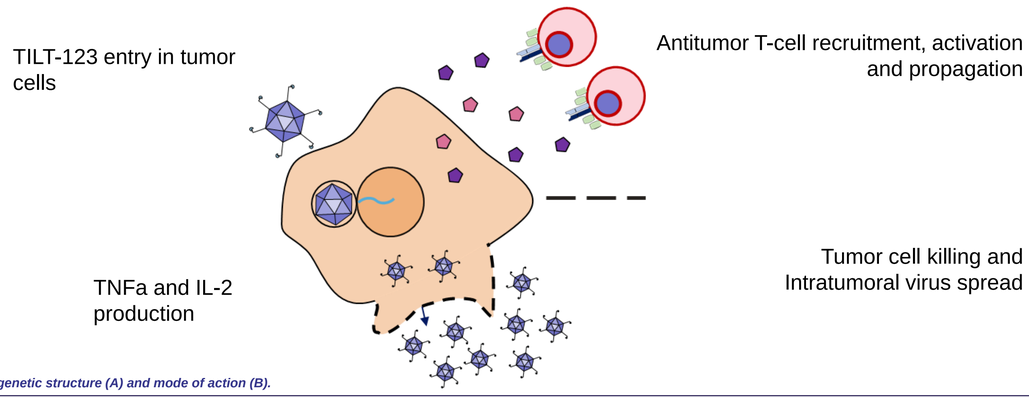
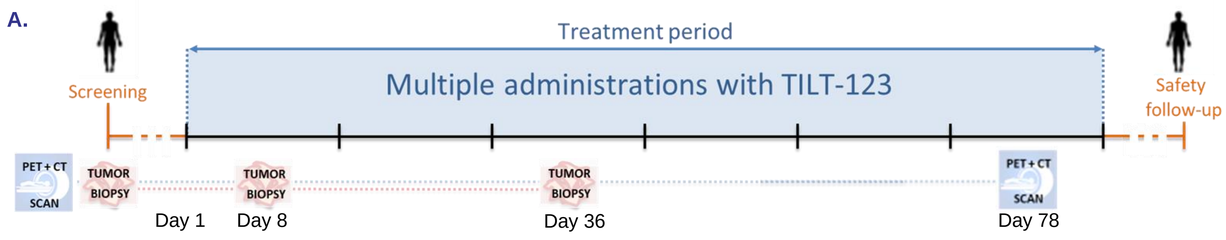


Figure 1 – TILT-123 genetic structure (A) and mode of action (B).

Trial Design

- **TUNIMO (TILT-T115; NCT04695327)** is an open label phase I clinical trial using a standard 3+3 dose-escalation scheme;
- Treatment is given to participants, who have advanced solid tumors, which are refractory to available therapies;
- TILT-123 was administered through intravenous (x1) and intratumoral/intravenous (at least x5) routes, throughout the trial.
- TUNIMO enrolled at 2 sites in Helsinki, Finland: Docrates Cancer Center and Helsinki University Hospital.

A.



B.

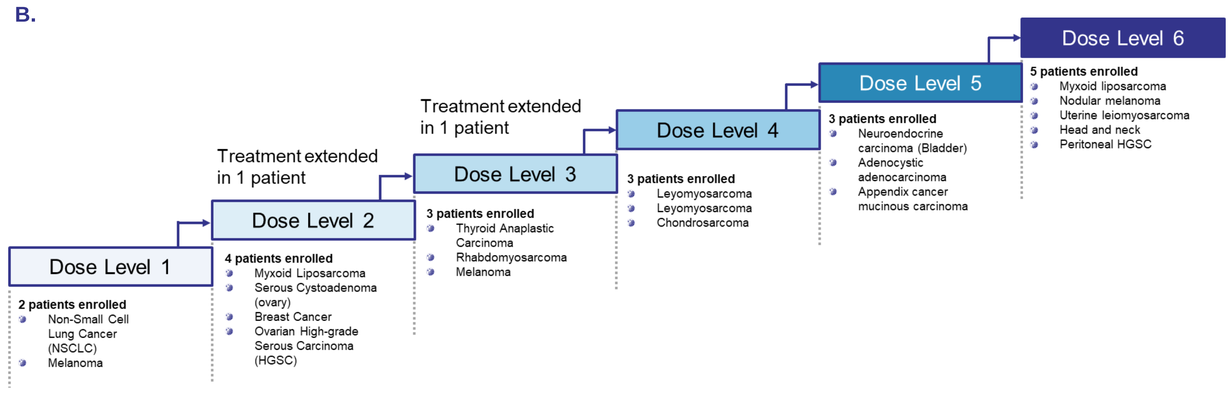


Figure 2 – Schedule of proceedures (A) dose-escalation scheme (B). As of 31st of August of 2023.

1 Demographics and Safety

Table 1 – Demographics of dose levels. As of 31st August 2023. *The results have not been fully monitored audited or verified by the quality assurance.*

Parameter	Total (n=20 pts)
Sex	
Female	13
Male	7
Age at Enrollment (Years)	
Median	58 (range 33-72)
Performance Status	
0	5
1	15
Tumor Type	
Sarcoma	7
Melanoma	3
Head and Neck	3
Ovarian and Peritoneal	3
Breast	1
Mucinous	1
Neuroendocrine	1
Lung	1
Prior Cancer therapies	
Median number of previous systemic treatment lines	4.5 (range 1-15)

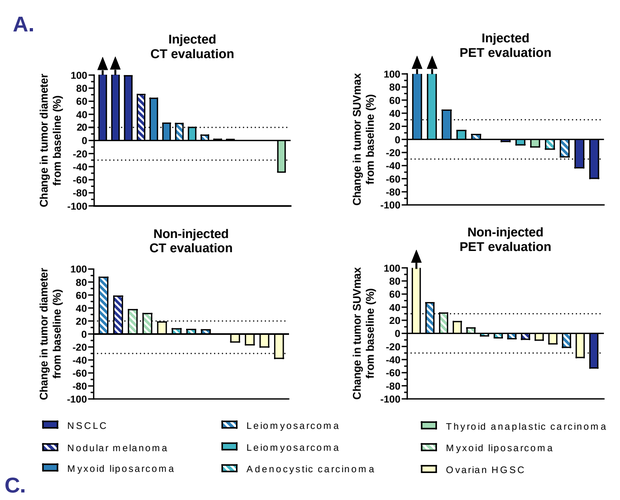
- Patients enrolled underwent several lines of therapy before enrolling into the trial (Table 1);
- WHO/ECOG performance status was mostly 1 (Table 1);
- Most common types of cancers in dose levels were Sarcoma, Melanoma, Ovarian and peritoneal cancer and head and neck (Table 1);
- The most frequent AEs observed were fever, chills and fatigue (Table 2);
- Dose-limiting toxicities **were not observed** (Table 2).

Table 2 – TILT-123-related adverse events as judged by the investigators. As of 31st August 2023. *Adverse events graded based on the Common Terminology Criteria for Adverse Events (CTCAE). The results have not been fully monitored audited or verified by the quality assurance.*

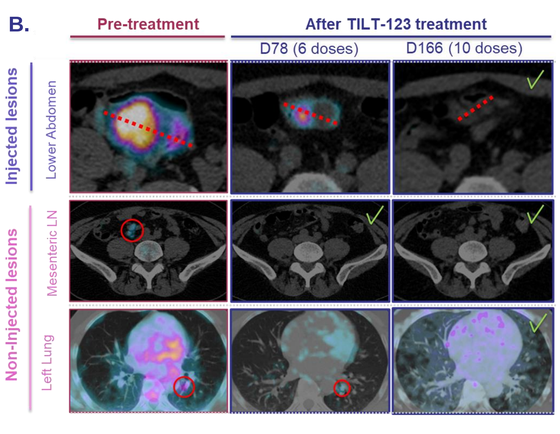
Preferred Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Cumulative Event Number (any grade)
Fever	**14**	4	0	0	0	**18**
Chills	**13**	1	0	0	0	**14**
Muscle pain	**4**	1	0	0	0	**5**
Subfebrile body Temperature	**3**	0	0	0	0	**3**
Flu-like symptoms	**1**	0	1	0	0	**2**
Fatigue	5	**4**	1	0	0	**10**
Tiredness	**3**	3	0	0	0	**6**
Nausea	**1**	**1**	0	0	0	**2**
Dry Mouth	**1**	0	0	0	0	**1**
Headache	1	**0**	0	0	0	**1**
Hot waves	1	**0**	0	0	0	**1**
Pain in Hands and Legs	**1**	0	0	0	0	**1**
Tinnitus	**1**	0	0	0	0	**1**
Neutropenia	**0**	1	4	1	0	**6**
Leukopenia	**0**	4	1	0	0	**5**
Thrombocytopenia	**1**	1	0	1	0	**3**
Lymphopenia	**0**	1	1	0	0	**2**
Creatinine Increased	**1**	1	0	0	0	**2**
Diarrhea	**1**	1	0	0	0	**2**
Loss of Apetite	**2**	0	0	0	0	**2**
Vomiting	**2**	0	0	0	0	**2**
Edema in feet	**1**	0	0	0	0	**1**
Pain or cramps in legs	**2**	0	0	0	0	**2**
Pain in joints	**2**	0	0	0	0	**2**
Worsening of pain in left knee	**0**	1	0	0	0	**1**
Cytokine Release Syndrome	**1**	1	0	0	0	**2**
Pain in Tumor	**5**	4	0	0	0	**9**
Infection in Tumor	**0**	1	0	0	0	**1**
Sweling of Metastasis	**1**	0	0	0	0	**1**
Total	**68**	**30**	**8**	**2**	**0**	**108**

2 Anti-Tumor Activity

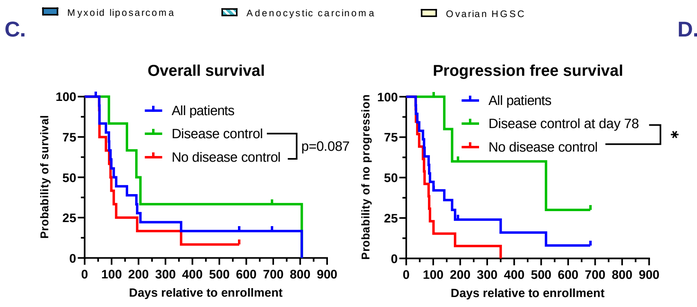
A.



B.



C.



D.

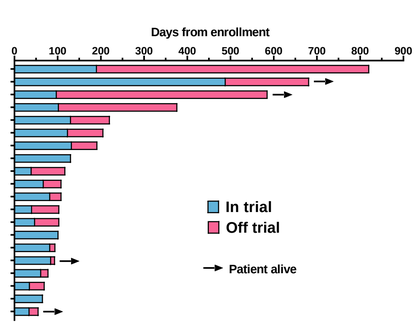
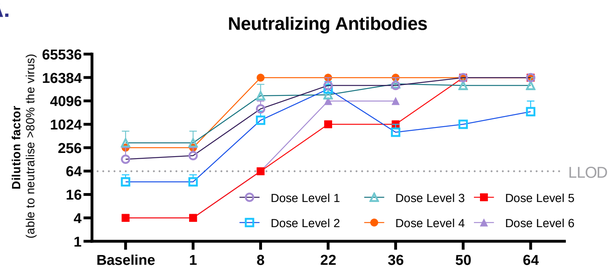


Figure 3 –Change in tumor size by Computerized Tomography (CT) scans and metabolic activity per SUV_{max} by Positron Emission Tomography (PET) scans in injected and non-injected tumors (A), example of anti-tumor activity in a patient with thyroid cancer (B), overall survival and progression free survival in treated patients (C), duration of trial for patients enrolled (D). *The results have not been fully monitored audited or verified by the quality assurance.*

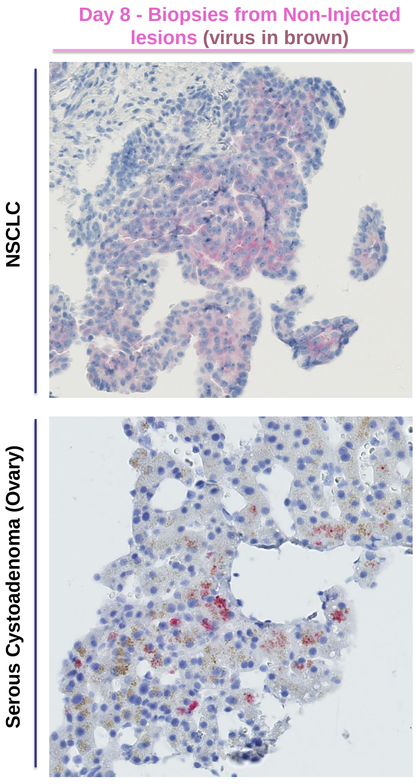
- 9 patients were evaluable for imaging by day 78.
- 67% of patients experienced disease control (SMD or better) by PET criteria
- Patient with thyroid cancer who was progressing before treatment
  - Patient entered treatment extension period;
  - The response deepened over time leading to the disappearance of non-injected lung and peritoneal lesions.
- Patients with disease control saw an overall survival and progression free survival of more than 25% (approximately 2 years)
- 4 patients off-trial are still alive

3 Biodistribution

A.



B.



C.

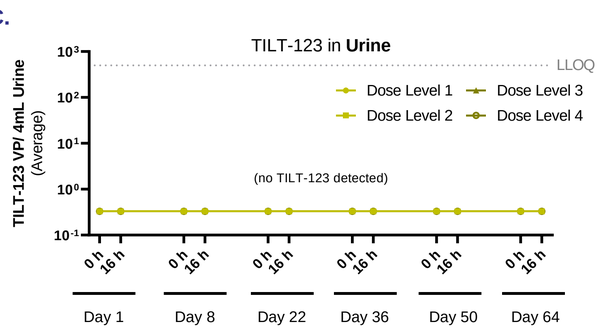
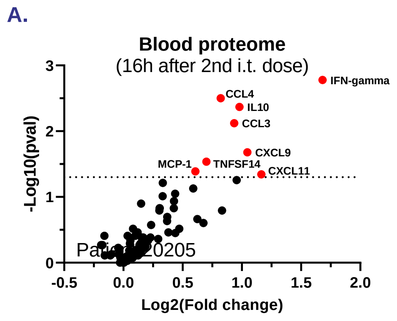


Figure 4 –Neutralizing antibodies in serum from treated patients (A), presence of TILT-123 in biopsies from non-injected tumors at day 8 (B), Kinetics of virus distribution in Urine (C)). *The results have not been monitored audited or verified by the quality assurance. LLOD – Lower Limit of Detection; LLOQ – Lower Limit of Quantification <500 VP / respective matrix volume. Average values of samples where no TILT-123 was detected (=0) were set to 0.33 in order to be visibly represented in the log scales of C. and D. graphs.*

- Analyzed sera from TILT-123-treated patients (n=12) showed an increase in neutralizing antibodies (Figure 4A);
- Most patients (n=7) had undetectable levels of neutralizing antibodies against TILT-123 at baseline (Figure 4A);
- Virus can be found in non-injected tumors after IV injection of TILT-123 in a patient from dose-level 1 (NSCLC) and another from dose level 2 (Serous Cystoadenoma (ovary)) (Figure 4B);
- Analyzed urine samples from TILT-123-treated patients (n=10) showed no virus genomes detected (Figure 4C);

4 Immunological activity

A.



B.

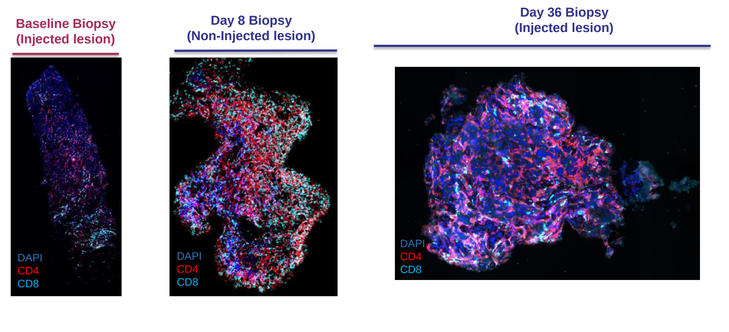


Figure 5 – Changes of the sera proteome after 2nd intratumoral injection (A), example of immune cell infiltration in a tumor from a patient treated demonstrated by multiplex immunohistochemistry (B). *The results have not been monitored audited or verified by the quality assurance. Multiplex immunohistochemistry images not in the same zoom scale.*

- Levels of pro-inflammatory cytokines (eg. IFNg, CXCL9, CXCL11) increase after intravenous and intratumoral injections (Figure 5A);
- Treatment with TILT-123 induced the intratumoral trafficking of CD4 and CD8+ T-cells (Figure 5B);

Conclusions

- This dose-escalation study indicates that TILT-123 is safe in humans;
- The safety profile observed, is consistent with other virus-based immunotherapies;
- Treatment with TILT-123 can enable anti-tumor responses as monotherapy, which can be seen in injected and non-injected lesions;
- TILT-123 can reach tumors and deliver efficacy despite neutralizing antibodies.
- Shedding of TILT-123 genomes form analyzed urine samples was not detected;
- Administration of TILT-123 triggers a systemic inflammatory response and enriches the tumor microenvironment with T-cells.

For more information contact:

João Manuel Santos
Head of Immunology at TILT Biotherapeutics
Email: joao.santos@tiltbio.com

We thank the patients and their families, and staff at:

HUS Helsinki University Hospital
DOCRATES CANCER CENTER

Study sponsored by:

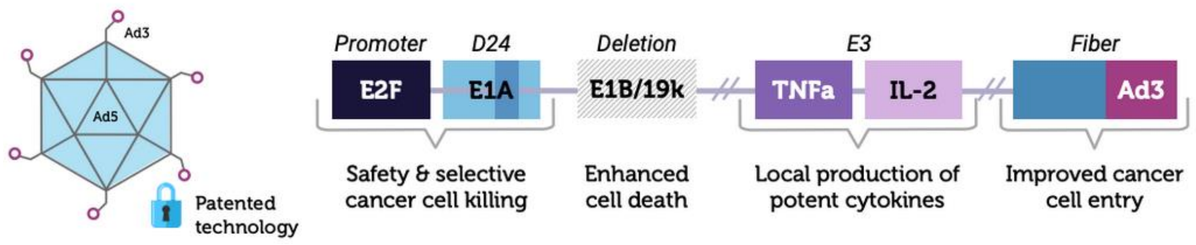
https://tiltbio.com/

Emerging proteomic and safety analysis of blood from patients receiving TILT-123 (Ad5/3-E2F-d24-hTNFa-IRES-hIL2) monotherapy in TUNIMO phase 1 clinical trial

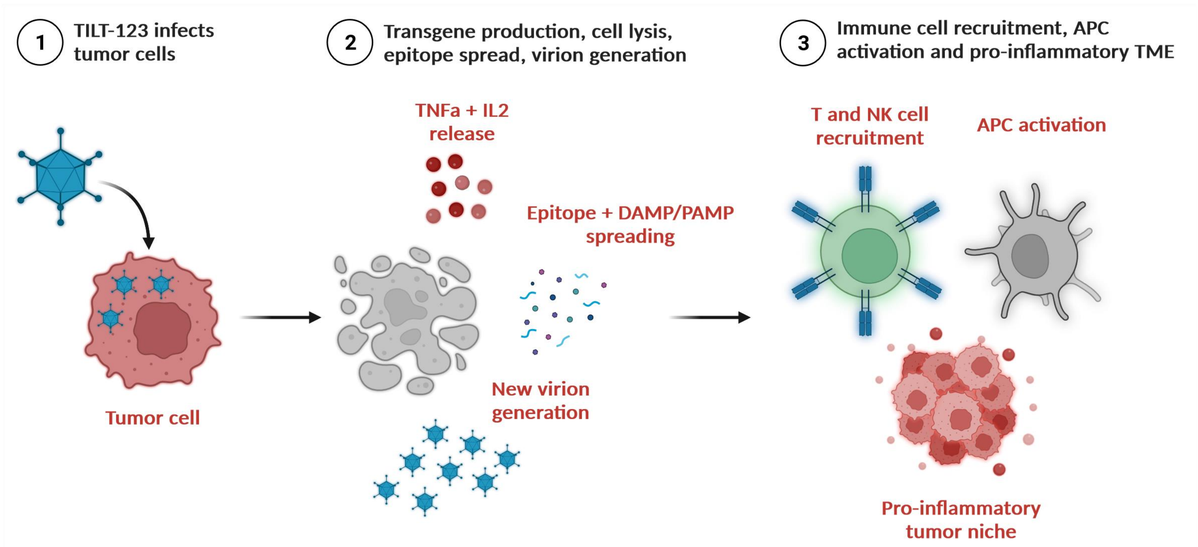
S. Pakola[1], V. Cervera-Carrascon[1,2], K. Peltola[3], T. Alanko[4], R. Korpisaari[4], M. Jaakkola[4], J. Sormunen[4], J. Kononen[4], J. Clubb[1,2], E. Jirovec[1], T. Kudling[1], L. Haybout[1,2], D.C.A. Quixabeira[1,2], C. Kistler[2], J.M. Santos[1,2], S. Sorsa[1,2], R. Havunen[1,2], A. Hemminki[1,2,3]

1) Cancer Gene Therapy Group, Translational Immunology Research Program, University of Helsinki, Helsinki, Finland. 2) TILT Biotherapeutics Ltd, Helsinki, Finland. 3) Comprehensive Cancer Center, Helsinki University Hospital, Helsinki, Finland. 4) Docrates Cancer Center, Helsinki, Finland.

BACKGROUND

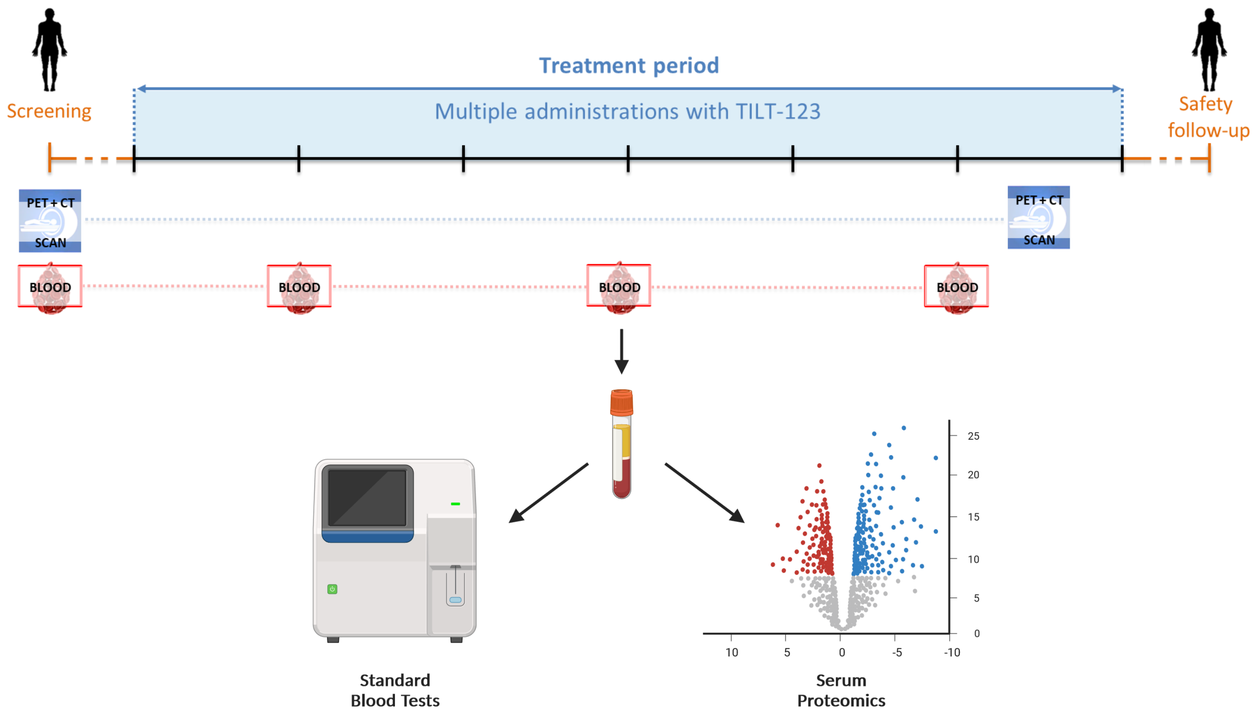


- TILT-123 is an oncolytic adenovirus armed with tumor necrosis alpha (TNFa) and interleukin-2 (IL-2).
- TILT-123 was desinged to induce T cell infiltration into solid tumors and boost their effector function.



- TILT-123 is currently evaluated in 4 clinical trials: TUNIMO (advanced solid cancers, monotherapy, NCT04695327), TUNINTIL (melanoma, with TIL therapy, NCT04217473), PROTA (ovarian cancer, with pembrolizumab, NCT05271318) and AVENTIL (anti-PD(L)1 resistant melanoma/SCCHN, with avelumab, NCT05222932).

METHODS



- TILT-123 was administered intravenously and intratumorally in the TUNIMO trial and blood was collected prior and after therapy administration.
- Blood was utilized for standard laboratory testing and serum extraction followed by Olink proteomics analysis.

COI & ACKNOWLEDGEMENTS & CONTACT

The presenting author has no COI to declare. TILT Biotherapeutics provided study material and reagents.

We like to thank the patients, families and hospital staff taking part in the TUNIMO study.

Santeri PAKOLA

Doctoral Researcher (MD-PhD Program) at Cancer Gene Therapy Group

University of Helsinki, Finland

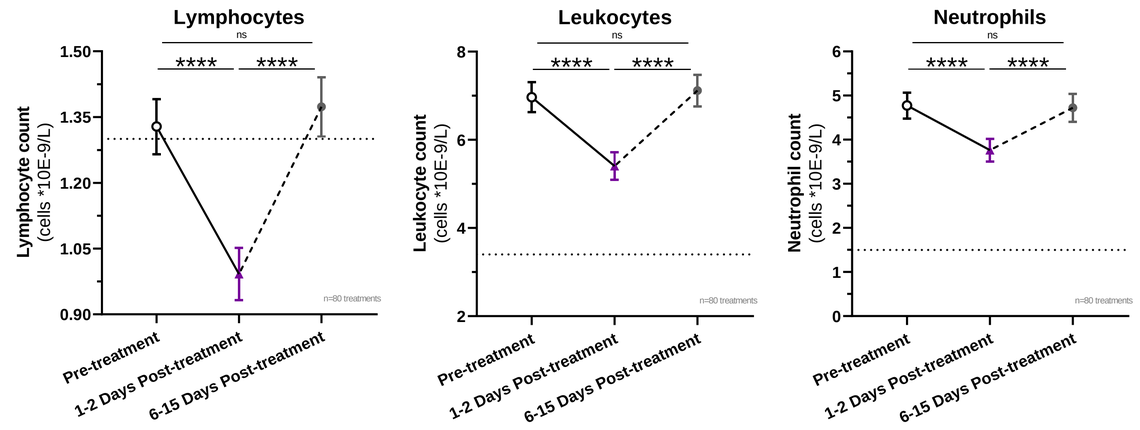
santeri.pakola@helsinki.fi

CONCLUSIONS

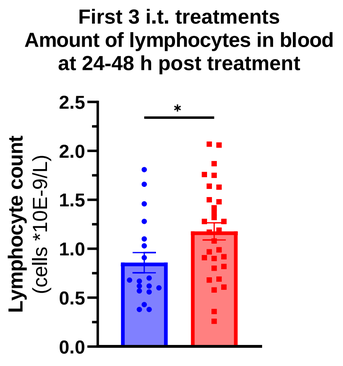
1. **TILT-123 induces decrease in total lymphocytes indicative of sequestration into tumors and this correlates with response.**
2. **No clinically relevant decrease in neutrophils or total leukocytes.**
3. **Pro-inflammatory changes in the serum are seen after intravenous and intratumoral administration.**
4. **Repeated intratumoral dosing produces stronger serum proteomic changes – lymph node or tumor niche priming?**

RESULTS

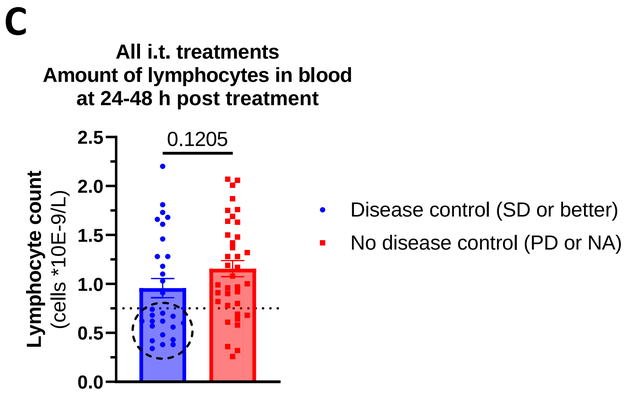
A



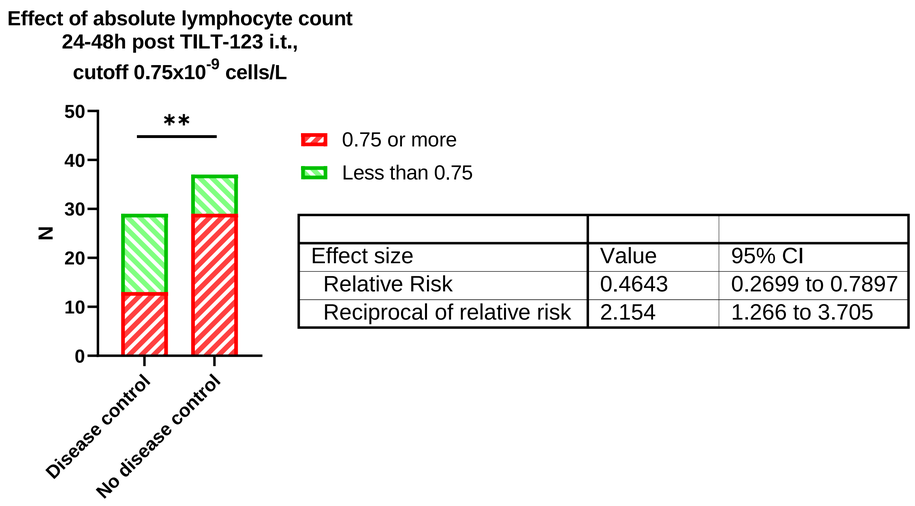
B



C



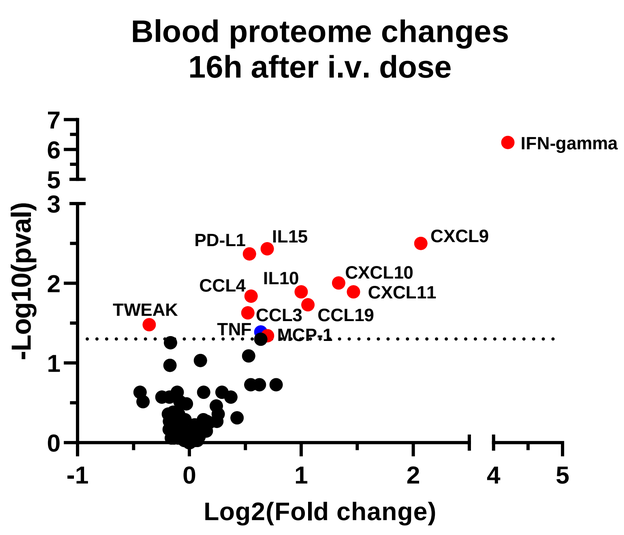
D



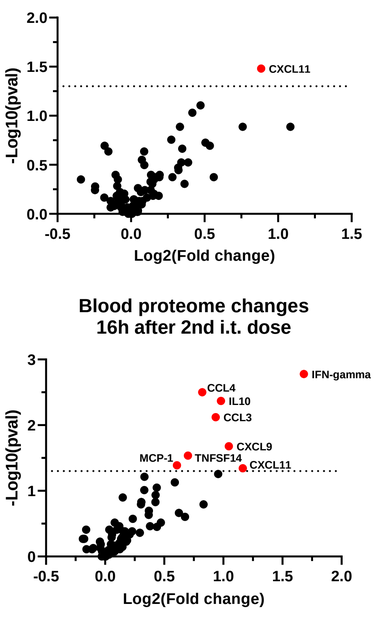
Effect size	Value	95% CI
Relative Risk	0.4643	0.2699 to 0.7897
Reciprocal of relative risk	2.154	1.266 to 3.705

- TILT-123 administration induces predominantly lymphocyte decrease acutely in blood. (**A**)
- Lower lymphocyte count post TILT-123 treatment correlates with treatment efficacy when looking at first three i.t. administrations. (**B**)
- Lymphocyte count less than 0.75 x 10^{-9} /L correlates with better response in all intratumoral administrations. (**C** and **D**)

E



F



- TILT-123 induces pro-inflammatory changes in the serum. (**E**)
- 2nd intratumoral administration produces stronger changes than 1st administration – priming of tumor niche or lymph nodes? (**F**)

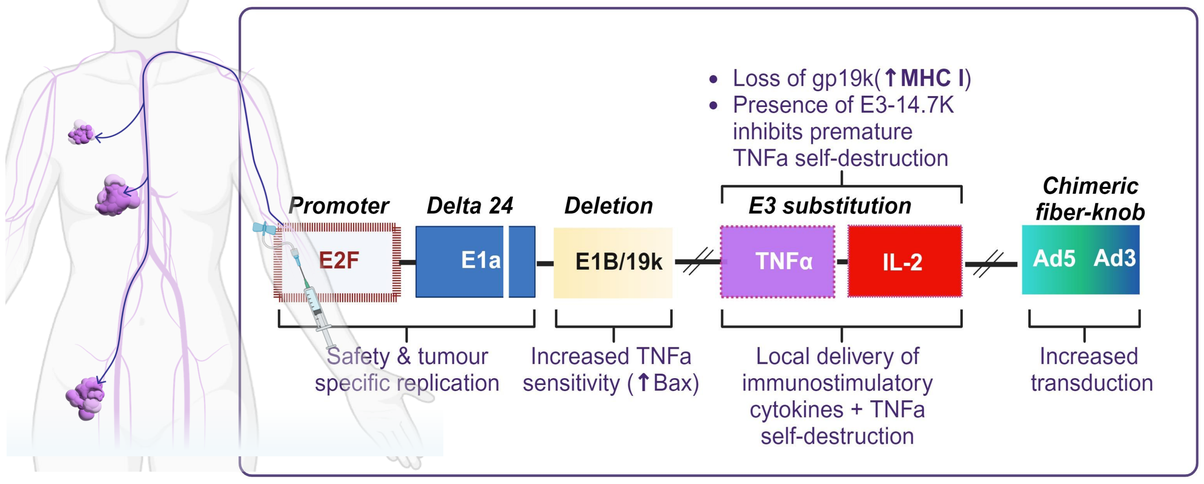
Oncolytic adenovirus encoding TNFα and IL-2 (igrelimogene litadenorepvec; TILT-123) from preclinical development to Phase I clinical trials and beyond.

J. H.A.Clubb[1,2], T. Kudling[2], J.M Santos[1,2], D.C.A. Quixabeira[1,2], K. Peltola[3], T. Alanko[4], S. Pakola[2], E. Jirovec[2], L. Haybout[1,2], N.E.Salmelin , V. Arias[1], S. Basnet[1], R. Korpisaari[4], S. Juteau[5], M. Jaakkola[5], J. Sormunen[4], J. Kononen[4], C. Kistler[1], S. Sorsa[1,2], R. Havunen[1,2], V. Cervera-Carrascon[1,2], A. Hemminki[1,2,3].

1) TILT Biotherapeutics Ltd, Helsinki, Finland, 2) Cancer Gene Therapy Group, Translational Immunology Research Program, University of Helsinki, Helsinki, Finland , 3) Comprehensive Cancer Centre, Helsinki University Hospital, Helsinki, Finland, 4) Docrates Cancer Center, Helsinki, Finland, 5) HUS Helsinki University Hospital

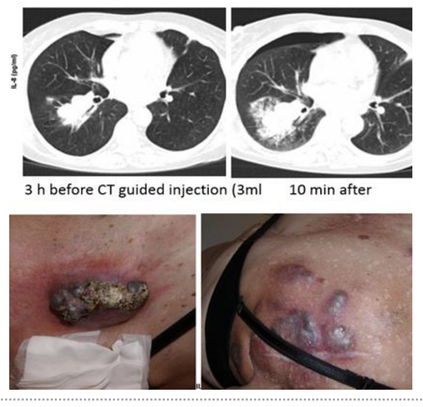
Background

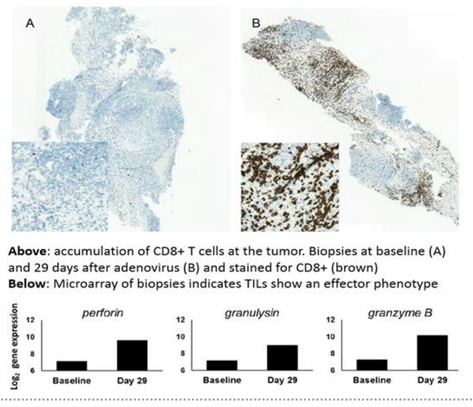
- Recent clinical trials evaluating the potential of oncolytic virotherapy has revealed encouraging results, giving hope to patients with incurable advanced stage cancer.
- Igrelimogene litadenorepvec (Ad5/3-E2F-d24-hTNF-IRES-hIL2; TILT-123), is an oncolytic adenovirus encoding for interleukin-2 and tumor necrosis factor alpha, designed for recruiting, propagating and stimulating T-cells and boosting the efficacy of existing T cell therapies such as immune checkpoint inhibitors and adoptive cell therapy.
- After almost two decades of development, TILT-123 is now in multiple Phase I clinical trials around the world, for the treatment of different solid tumor indications. Promising clinical responses have been observed and analysis of samples proceeds with the hope of identifying biomarkers to improve patient stratification. However, additional ongoing preclinical characterization has also revealed novel insights which may guide better exploitation of this promising therapy.



Advanced-Therapy Access Program and preclinical development

Compassionate treatment program in Finland showcases potential of oncolytic adenoviruses and reveals insights to guide preclinical development of TILT-123.

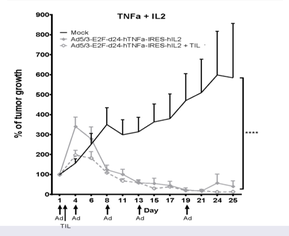




Above: accumulation of CD8+ T cells at the tumor. Biopsies at baseline (A) and 29 days after adenovirus (B) and stained for CD8+ (brown)
Below: Microarray of biopsies indicates TILs show an effector phenotype

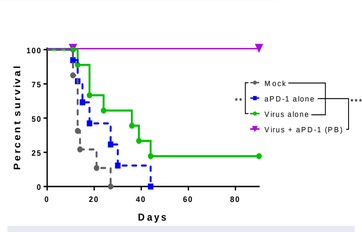
Preclinical development, beginning with a recombinant cytokine screen of FDA approved cytokines reveals IL-2 and TNFα as superior for enhancing adoptive cell therapy. These cytokines were later loaded into the Ad5/3-d24-E2F backbone and TILT-123 was born. Preclinical studies later reveal complete responses in multiple tumor models.

Enables **TILs**



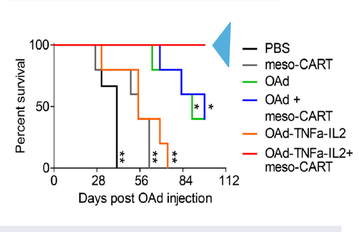
100% Overall Survival
(Havunen R. Mol Ther 2016)

Enables **ICIs**



100% Overall Survival
(Cervera-Carrascon V. Oncoimmunology 2018)

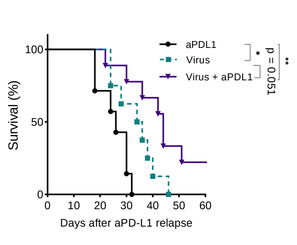
Enables **CAR T**

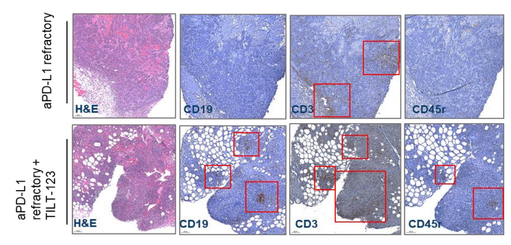


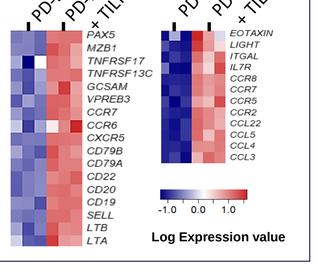
100% Overall Survival
(Watanabe K. JCI 2018)

1 TILT-123 induces tertiary lymphoid structure formation

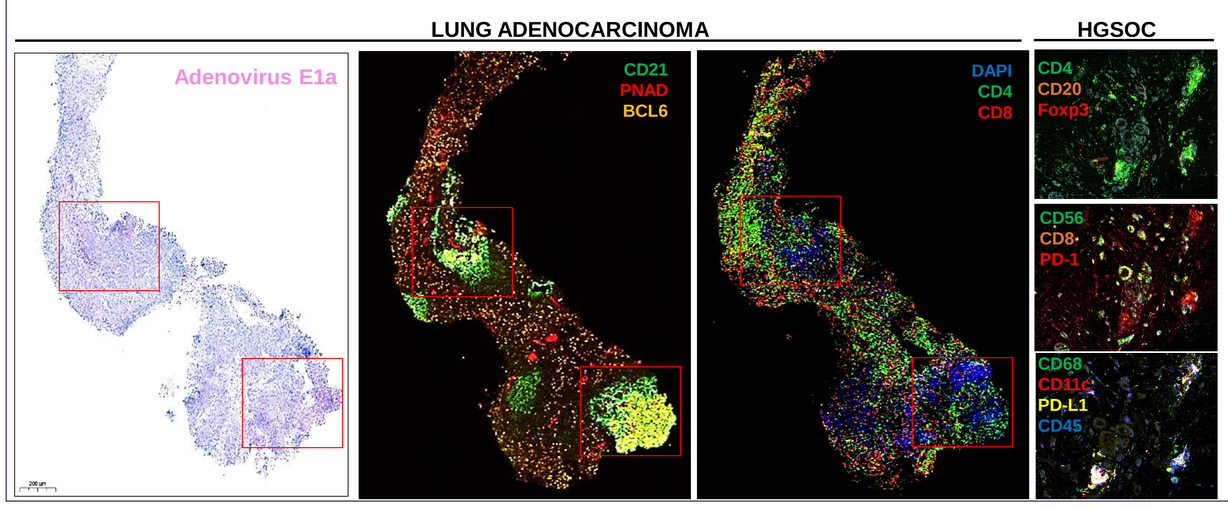
Preclinical study in mice demonstrates TILT-123 induces tertiary lymphoid structure formation and re-sensitisation of tumours to PD-L1 in a mouse model of PD-L1 refractory SCCHN.







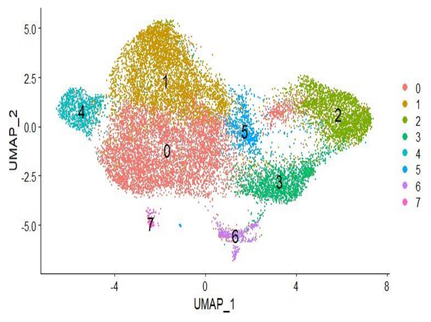
Biopsies taken from patients tumours after treatment with TILT-123 shows localisation of virus E1a with induction of tertiary lymphoid structures.



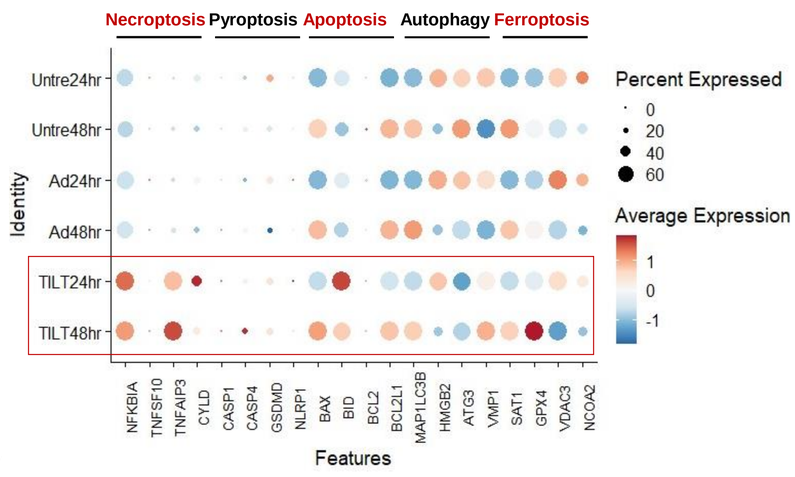
2 TILT-123 induces immunogenic cell death in LUAD

Analysis of TILT-123 induced cell death pathways using single cell RNA-Seq and western blot

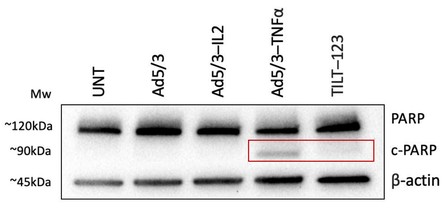
WT scRNA-Seq of TILT-123 infected LUAD cell line *in vitro* reveals distinct clusters



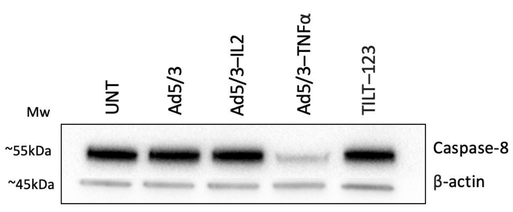
TILT-123 induces distinct cell death profiles



PARP cleavage induced by TILT-123 derived TNFα (sensitization)

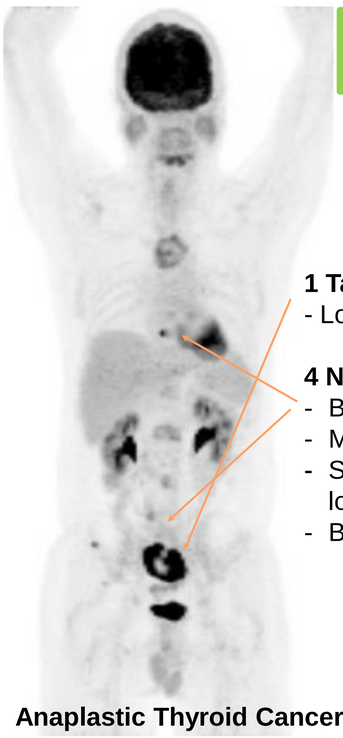


TILT-123 derived IL-2 inhibits caspase 8 cleavage induced by TNFα



3 Effective elimination of metastatic lesions as monotherapy

- **TILT-T115 (TUNIMO; NCT04695327)** is an open label phase I clinical trial using a standard 3+3 dose-escalation scheme;
- Treatment is given to participants, who have advanced solid refractory to available therapies;
- TILT-123 was administered through intravenous and intratumoral routes, throughout the trial.
- TILT-T115 is currently enrolling at 2 sites in Helsinki, Finland: Docrates Cancer Center and Helsinki University Hospital.

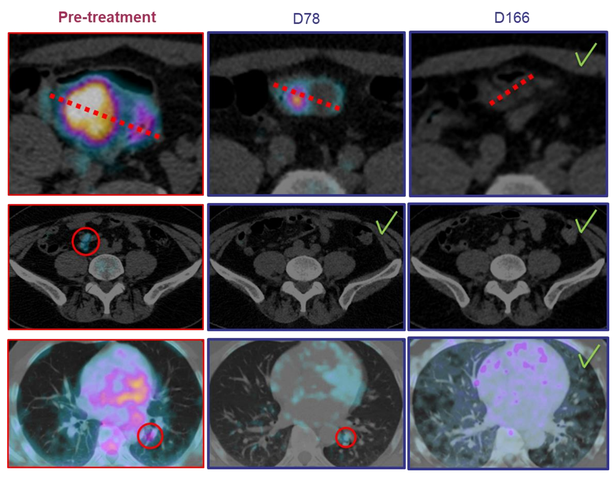


Anaplastic Thyroid Cancer

PARTIAL RESPONSE

1 Target lesion:
- Lower abdomen

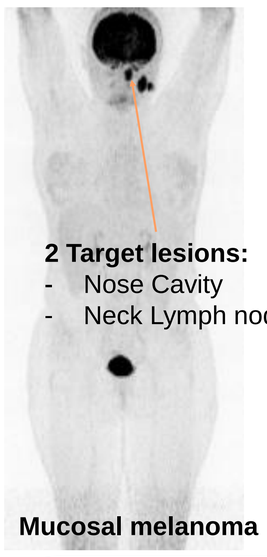
4 Non-target lesions:
- Bone lysis sternum
- Mesenteric LN
- Solitary lung lower lobe
- Bone lysis lumber



- 70% reduction in injected target lesion as measured by CT value 166 days after first treatment.
- 100% reduction of non-injected metastatic lesions.as measured by CT value 166 days after first treatment.

4 Complete response in combination with TILs in melanoma

- **TILT-T215 (TUNTIL; NCT04217473)** is an open label phase I clinical trial using TILT-123 in Melanoma patients receiving adoptive cell therapy with tumour infiltrating lymphocytes.
- Treatment is given to participants, who have advanced solid refractory to available therapies;
- TILT-123 was administered through intravenous and intratumoral routes, throughout the trial.
- TILT-T115 is currently enrolling at 2 sites in France and Herlev, Denmark

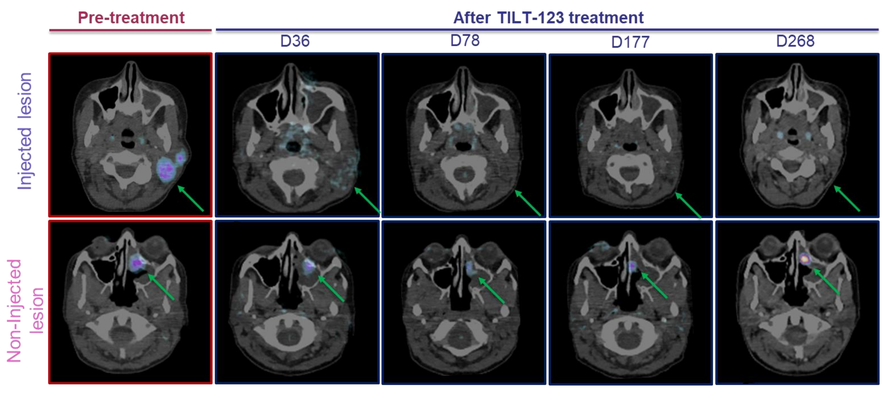


Mucosal melanoma

2 Target lesions:
- Nose Cavity
- Neck Lymph node

PATHOLOGICAL COMPLETE RESPONSE

- Non-injected nose lesion was later operated on and no cancer cells were found.



For more information contact:

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

We thank the patients and their families, and staff at:

HUS Helsinki University Hospital
DOCRATES CANCER CENTER

Ida Montinin Säätiö PERUSTETTU VUONNA 1971
ORION
Syöpäsäätiö Cancer Foundation Finland
MINERVA FOUNDATION
K. Albin Johanssons Stiftelse
ACADEMY OF FINLAND

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

Study sponsored by:

https://tiltbio.com/

Immune cell profiling of advanced-stage solid tumors patients treated with an oncolytic adenovirus encoding for TNF-a and IL-2 (TILT-123)

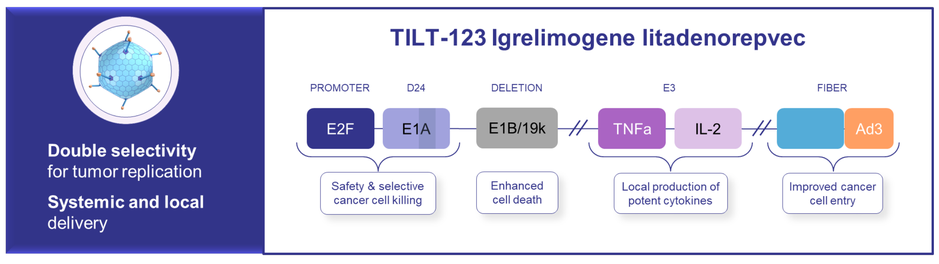
D. C. A. Quixabeira 1,2, S. Pakola 2,5, J. Clubb 1,2, L. Haybout 1,2, E. Jirovec 2, T. Kudling 2, J.M. Santos1,2, V. Cervera-Carrascon 1,2, K. Peltola 3, T. Alanko4, J. Sormunen4, R. Korpisaari4, M. Jaakkola5, J. Kononen4, S. Juteau6, C. Kistler1, S. Sorsa1,2, R. Havunen1,2, A. Hemminki1,2,3.

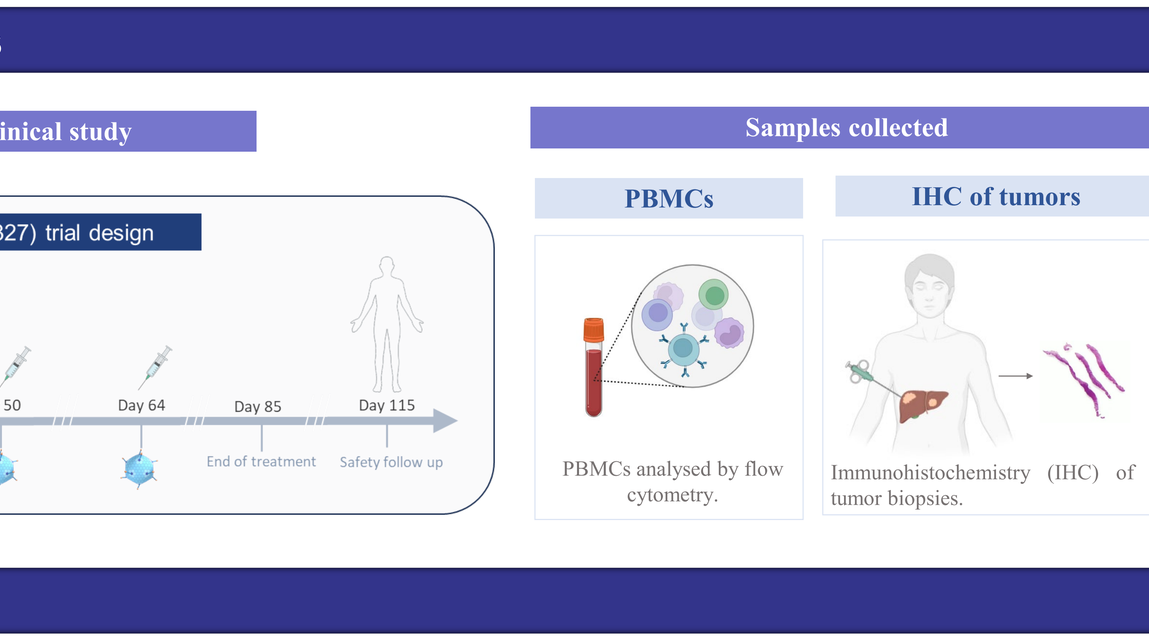
1) TILT Biotherapeutics Ltd, Helsinki, Finland, 2) Cancer Gene Therapy Group, Translational Immunology Research Program, University of Helsinki, Helsinki, Finland, 3) Comprehensive Cancer Center, Helsinki University Hospital, Helsinki, Finland, 4) Docrates Cancer Center, Helsinki, Finland 5) HUS Helsinki University Hospital, 6) Department of Pathology, Helsinki University Hospital, Helsinki, Finland

745

Background

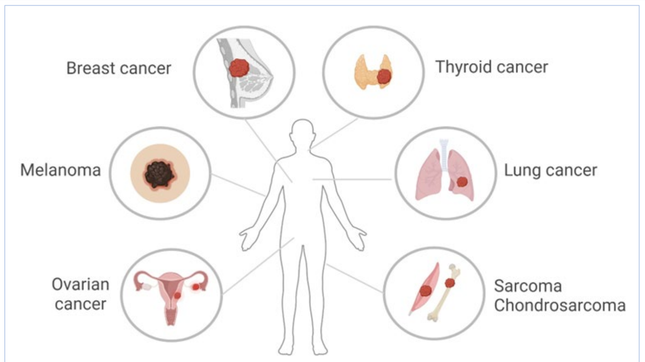
- Stimulating efficient anti-tumor response in poorly immune-infiltrated tumors is yet a challenge for currently approved immunotherapeutic drugs;
- Emerging immunotherapies, such as oncolytic viruses that have the potential to overcome tumor immunosuppression and stimulate immune cells' response;
- TILT-123, a genetically modified oncolytic adenovirus expressing TNFa and IL-2 cytokines (Ad5/3-E2F-D24-hTNFa-IRES-hIL2), has shown potent efficacy in a series of pre-clinical studies as a monotherapy and as an enabler of clinically used immunotherapies such as checkpoint inhibitors and cell therapies.
- In the present work, TILT-123 is being tested in a phase I clinical trial as a monotherapy for the treatment of advanced-stage human solid tumors;



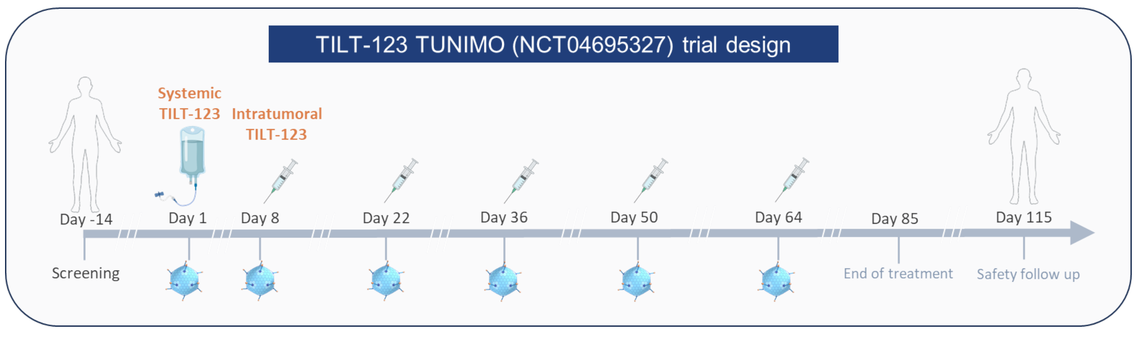


Methods

Refractory or recurrent solid tumors, which cannot be treated with curative intent with available therapies



TILT-123 monotherapy clinical study



Samples collected

PBMCs

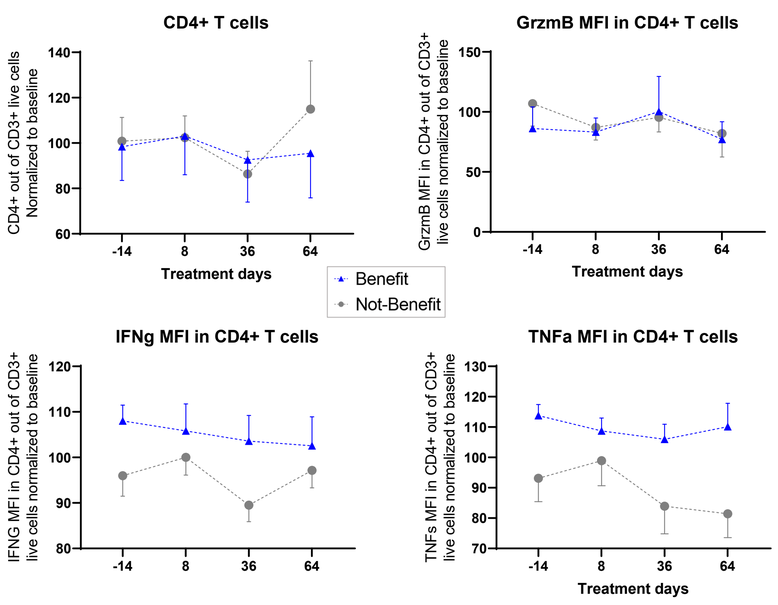
PBMCs analysed by flow cytometry.

IHC of tumors

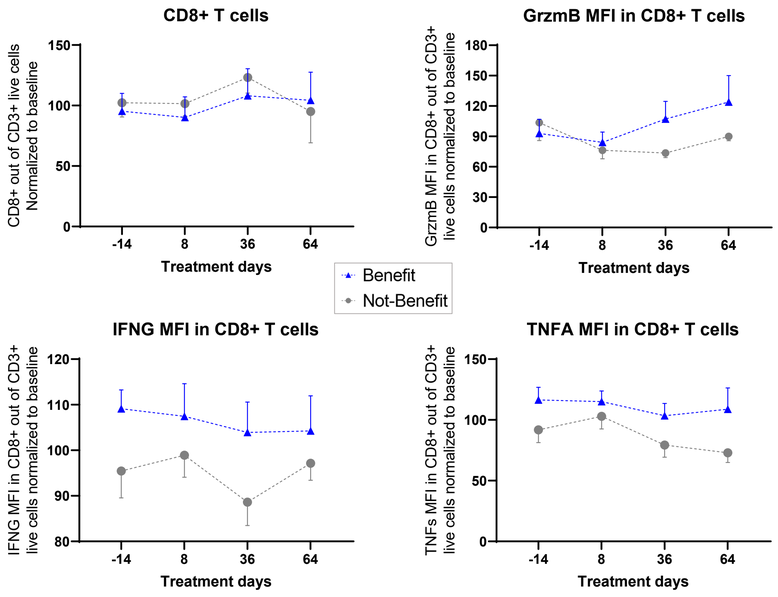
Immunohistochemistry (IHC) of tumor biopsies.

Results

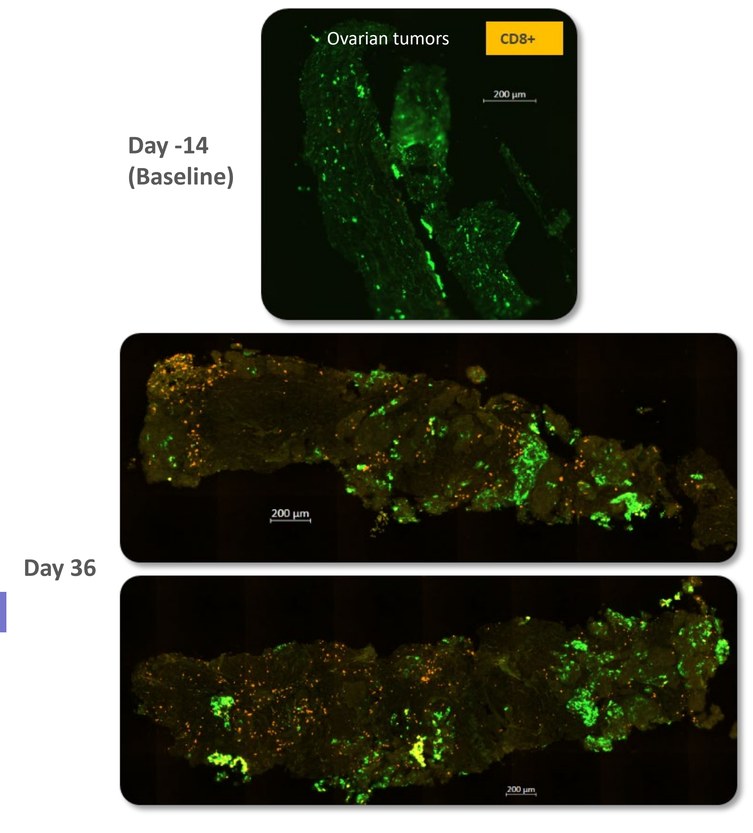
CD4+ T cells changes in peripheral blood of patients treated with TILT-123



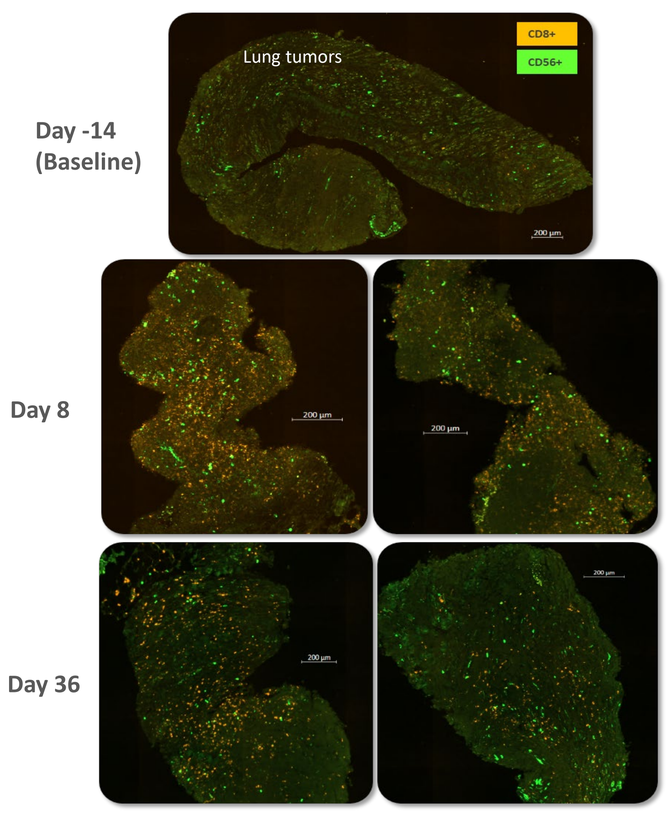
CD8+ T cells changes in peripheral blood of patients treated with TILT-123



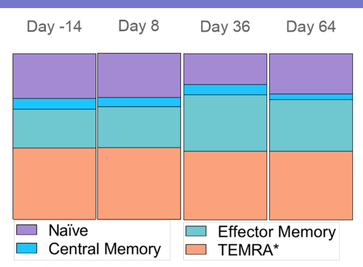
Systemic TILT-123 effect in CD8+ cells infiltrating non-injected tumors



Changes in the intratumoral infiltration of CD8+ and CD56+ cells after TILT-123 treatment

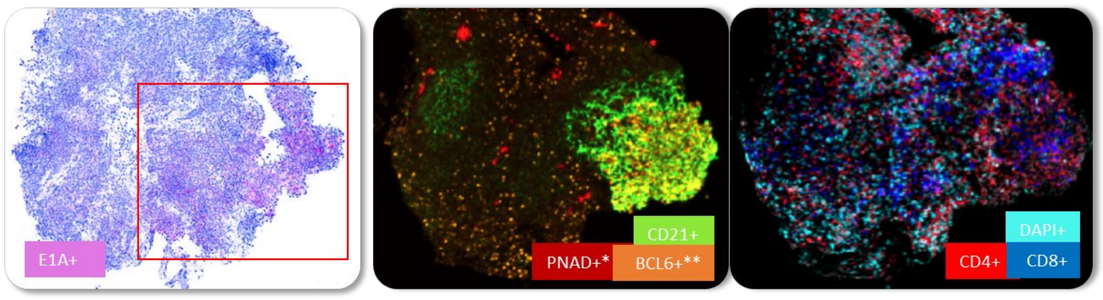


Memory CD8+ T cells in the peripheral blood of patients treated with TILT-123



*T effector memory re-expressing CD45A.

Colocalization of TILT-123 E1A viral protein and immune cells in a no-injected lung tumor



*High endothelial venules. **Transcription factor for T follicular Helper cells

Conclusions

Treatment with TILT-123 oncolytic adenovirus changes the profile of immune cells circulating systemically and locally infiltrating injected and non-injected tumor sites. For an overview of TILT-123 virus development and trial safety profile and patient response, please check posters **711**, **739**, **749**, and **1518**.

For more information contact:

Dafne Quixabeira
TILT Biotherapeutics
Email: *dafne.quixabeira@tiltbio.com*

For more information on TILT´s clinical trials, check our website:
https://tiltbio.com/

Follow our updates on LinkedIn:
https://fi.linkedin.com/company/tilt-biotherapeutics-ltd

We thank the patients and their families, and staff at:

Acknowledgments:

Study sponsored by: