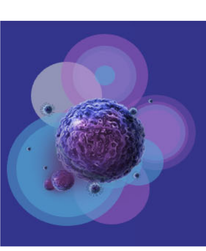

TILT-123, an oncolytic adenovirus encoding tumor necrosis factor alpha (TNFa) and interleukin (IL-2), for immunotherapy of solid tumors – Experience from phase I clinical trials

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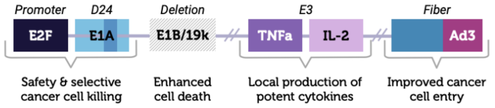
Background

- 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;
- TILT-123, an chimeric serotype oncolytic adenovirus encoding tumor necrosis factor alpha (TNFa) and Interleukin-2 (IL-2), is currently being tested in four phase I clinical trials, as monotherapy, and in combination with adoptive cell therapy or immune checkpoint inhibitors.

A.



B.

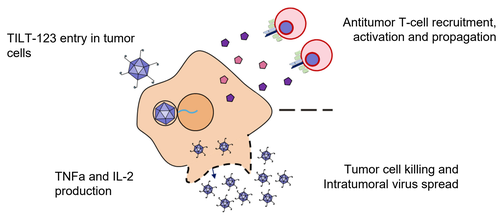


Figure 1 – TILT-123 genetic structure (A) and mode of action (B).

Trial Design

TILT-T115 (TUNIMO) - NCT04695327

- Trial 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 that cannot be treated with curative intent;
- TILT-123 is being 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.

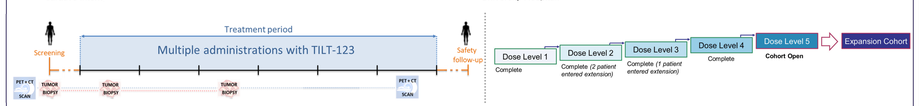


Figure 2 – TILT-T115 Clinical trial design and progress. Progress cut-off 29th August 2022.

Primary endpoint	Secondary endpoints	Exploratory Endpoints
TILT-123 safety by day 85	Dose-limiting Toxicities Efficacy [CT/PET imaging (RECIST 1.1, iRECIST); PFS, OS) Mode of Action (immune response to TILT-123) Maximum Tolerated Dose Pharmacokinetics/Biosafety	Biological effects in injected and non-injected tumors and blood, and gut microbiota Changes in skin lesions

TILT-T215 (TUNINTIL) - NCT04217473

- Trial is an open label, multinational, phase I clinical trial using a standard 3+3 dose-escalation scheme;
- Treatment is given to participants, who have metastatic melanoma that cannot be treated with curative intent;
- TIL Therapy is administered without pre- or post-conditioning in two different forms: one TIL infusion at Herlev Hospital, Denmark or two TIL infusions at CHU Nantes, France hospital;
- TILT-123 is being administered through intravenous and intratumoral routes at escalating doses, throughout the trial.
- TILT-T215 is currently enrolling at 2 sites in Europe: Herlev Hospital, Denmark and at the CHU Nantes, France.

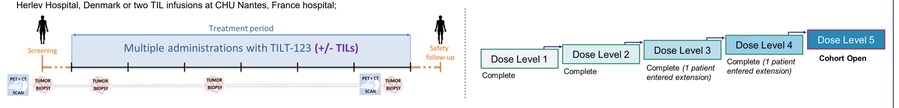


Figure 3 – TILT-T215 Clinical trial design and progress. Progress cut-off 30th August 2022.

Primary endpoint	Secondary endpoints	Exploratory Endpoints
TILT-123 safety by day 36	TILs + TILT-123 safety by day 78 Dose-limiting Toxicities Efficacy [CT/PET imaging (RECIST 1.1, iRECIST); PFS, OS) Mode of Action (immune response to TILT-123) Maximum Tolerated Dose Pharmacokinetics/Biosafety	Biological effects in injected and non-injected tumors and blood. Changes in skin lesions

1 Demographics and Safety

TILT-T115 (TUNIMO) - NCT04695327

Table 1 – Demographics of dose levels 1-3 . Data cut-off 29th of August 2022. *The results have not been monitored audited or verified by the quality assurance.*

Parameter	Total (n=9 pts)
Sex	
Female	6
Male	3
Age at Enrollment (Years)	
Median	60 (range 38-71)
Performance Status	
0	2
1	7
Tumor Type	
Lung	1
Melanoma	2
Sarcoma	2
Ovary	2
Breast	1
Thyroid	1
Prior Cancer therapies	
Median number of previous systemic treatment lines	5
Unlisted Therapies	55
Chemotherapy	35
Targeted Therapy	4
Combinations (e.g. chemo + targeted therapy)	4
Radiation Therapy	7
Surgery	8
Immunotherapy	5

Table 2 – TILT-123-related adverse events in dose levels 1-3 as judged by the investigators. Data cut-off 29th of August 2022. *Adverse events graded based on the Common Terminology Criteria for Adverse Events (CTCAE). The results have not been 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)	Event Frequency (any grade per patient)
Chills	3	1	0	0	0	4	22 %
Diarrhea	0	1	0	0	0	1	11 %
Edema in feet	1	0	0	0	0	1	11 %
Fatigue	2	1	0	0	0	3	22 %
Fever	6	0	0	0	0	6	33 %
Lymphopenia	0	1	0	0	0	1	11 %
Neutropenia	0	1	0	0	0	1	11 %
Pain in hands and legs	1	0	0	0	0	1	11 %
Subfebrile body temperature	1	0	0	0	0	1	11 %
Swelling of metastases	1	0	0	0	0	1	11 %
Tinnitus	1	0	0	0	0	1	11 %
Tiredness	0	1	0	0	0	1	11 %
Total	**16**	**6**	**0**	**0**	**0**	**22**	

- Patients enrolled underwent several lines of therapy before enrolling into the trial;
- WHO/ECOG performance status was mostly 1;
- Most common types of cancers in dose levels 1-3 were melanoma, Sarcoma and Ovary cancers;
- The most frequent AEs in dose levels 1-3 observed were fever, chills and fatigue;
- Grade 3-5 AEs **were not seen** in dose levels 1-3;
- Serious AEs **were not seen** in dose levels 1-3;
- Dose-limiting toxicties **were not seen** in dose levels 1-3.

TILT-T215 (TUNINTIL) - NCT04217473

Table 3 – Demographics of dose levels 1-3 . Data cut-off 30th of August 2022. *The results have not been monitored audited or verified by the quality assurance.*

Parameter	Total (n=9 pts)
Sex	
Female	2
Male	7
Age at Enrollment (Years)	
Median	48 (range 25-68)
Performance Status	
0	8
1	1
Melanoma Type	
Cutaneous	4
Ocular	2
Mucosal	2
Other	1
Prior Cancer therapies	
Median number of previous systemic treatment lines	3
(Not displayed therapies)	7
Chemotherapy	5
Targeted Therapy	2
Radiation Therapy	2
Surgery	10
Immunotherapy	24

Preferred Term	Grade 1	Grade 2	Grade 3	Grade 4	Grade 5	Cumulative Event Number (any grade)	Event Frequency (any grade per patient)
Alopecia	1	0	0	0	0	1	11 %
Anorexia	0	1	0	0	0	1	11 %
Arthralgia	1	0	0	0	0	1	11 %
Asthenia	2	1	0	0	0	3	33 %
Chills	1	0	0	0	0	1	11 %
Diarrhea	4	0	0	0	0	4	22 %
Dizziness	1	0	0	0	0	1	11 %
Edema, by left eye	1	0	0	0	0	1	11 %
Fatigue	3	0	0	0	0	3	22 %
Fever	5	2	0	0	0	9	56 %
Flushing	2	0	0	0	0	2	22 %
Headache	3	0	0	0	0	3	22 %
Itching at injection sites	1	0	0	0	0	1	11 %
Lymphopenia	1	0	0	0	0	1	11 %
Memory impairment	1	0	0	0	0	1	11 %
Myalgia	3	0	0	0	0	3	22 %
Nausea	2	2	0	0	0	4	33 %
Pain	1	0	0	0	0	1	11 %
Pain from injection	3	0	0	0	0	3	22 %
Pain in liver from injection	0	0	1	0	0	1	11 %
Pain, left knee	1	0	0	0	0	1	11 %
Severe myodesopsia at rest	1	0	0	0	0	1	11 %
TSH decreased	1	0	0	0	0	1	11 %
Vitiligo	1	0	0	0	0	1	11 %
Vomiting	1	0	0	0	0	1	11 %
Worsening of Headache	0	3	0	0	0	3	11 %
Total	**41**	**9**	**3**	**0**	**0**	**53**	

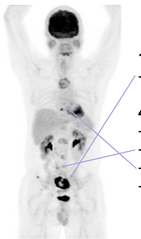
Table 4 – TILT-123-related adverse events in dose levels 1-3 as judged by the investigators. Data cut-off 30th of August 2022. *Adverse events graded based on the Common Terminology Criteria for Adverse Events (CTCAE). The results have not been monitored audited or verified by the quality assurance.*

- Patients enrolled were heavily pre-treated;
- WHO/ECOG performance status was mostly 0;
- Cutaneous Melanoma was the most prevalent cancer populaation in dose level 1-3;
- The most frequent Aes in dose levels 1-3 were fever, nausea and diarhea;
- Grade 3-5 AEs **were not seen** in dose levels 1-3;
- Serious AEs **were not seen** in dose levels 1-3;
- Dose-limiting toxicties **were not seen** in dose levels 1-3.

2 Anti-tumor Activity

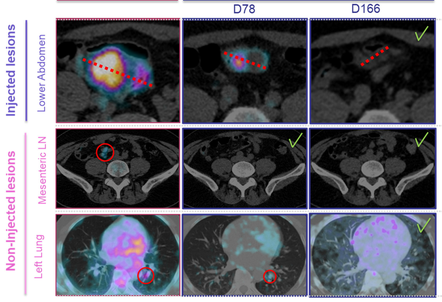
TILT-T115 (TUNIMO) - NCT04695327

Example #1 - Patient with Thyroid Cancer (Dose Level 3)



1 Target lesion:
- Lower abdomen

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



TL1	Pre-treatment	After TILT-123 (day 78)	After TILT-123 (day 166)
CT value	67 mm	34 mm (↓49%)	21 mm (↓69%)
SUVmax	18.5	16.2 (↓12%)	0 (↓100%)

NTL2	Pre-treatment	After TILT-123 (day 78)	After TILT-123 (day 166)
CT Value	Present	Absent	Absent

NTL4	Pre-treatment	After TILT-123 (day 78)	After TILT-123 (day 166)
CT Value	Present	Present	Absent

CT - Computed tomography

SUVmax - Maximum standardized uptake value from PET (positron emission tomography)

Overall Response (day 166)
PET
Minor Metabolic Response
RECIST 1.1
Partial Response

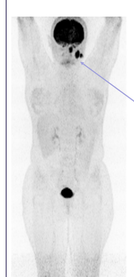
PET -Positron emission tomography; RECIST 1.1 – Response Evaluation Criteria in Solid Tumors 1.1

Figure 4 – Example of anti-tumor activity of a patient treated with TILT-123 as monotherapy. Data cut-off 29th of August 2022. *The results have not been monitored audited or verified by the quality assurance.*

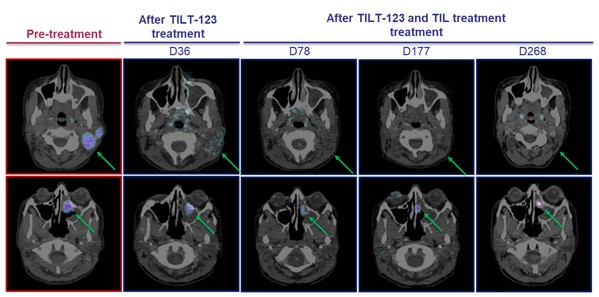
- Patient with thyroid cancer who was progressing before treatment;
- Patient entered treatment extension period;
- The response deepened over time leading to the disappearance of non-injected lung and peritoneal lesions.

TILT-T215 (TUNINTIL) - NCT04217473

Example #2 - Patient with Melanoma (Dose Level 3)



2 Target lesions:
- Nose Cavity
- Neck Lymphnode



		Pre-treatment	After TILT-123 treatment	After TILT-123 and TIL treatment		
			Day 36	Day 78	Day 177	Day 268
Neck lesion	CT value	20 mm	16 mm (↓20%)	10 mm (↓50%)	11 mm (↓45%)	11 mm (↓45%)
	SUVmax	16.9	5.7 (↓66%)	0 (↓100%)	0 (↓100%)	0 (↓100%)
Nose lesion	CT value	31 mm	28 mm (↓10%)	22 mm (↓29%)	20 mm (↓36%)	27 mm (↓12.9%)
	SUVmax	19.8	13.5 (↓32%)	5 (↓75%)	11.8 (↓40%)	22.2 (↑12.1%)

CT - Computed tomography

SUVmax - Maximum standardized uptake value from PET (positron emission tomography)

Overall Response (day 268)
PET
Complete Response*
RECIST 1.1
Complete Response*

* The lesion in the nose was operated on dX: no cancer cells. The neck lesion was biopsied on dY: no cancer. Therefore, the patient had pCR

PET -Positron emission tomography; RECIST 1.1 – Response Evaluation Criteria in Solid Tumors 1.1

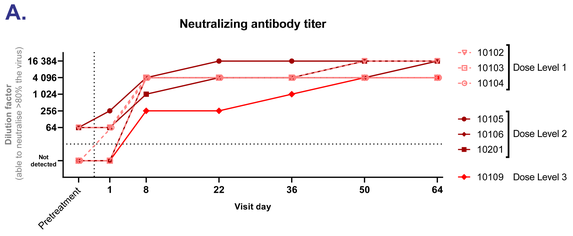
Figure 5– Example of anti-tumor activity of a patient treated with TILT-123 and TIL Therapy. Data cut-off 30th of August 2022. *The results have not been monitored audited or verified by the quality assurance.*

- Patient with melanoma that was progressing before treatment;
- Patient entered treatment extension period.

3 Neutralizing antibodies, biosafety and T-cell Infiltration

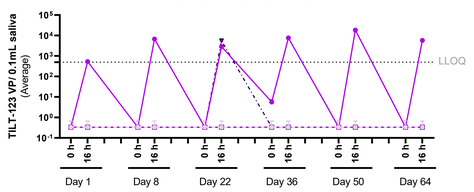
TILT-T215 (TUNINTIL) - NCT04217473

A.



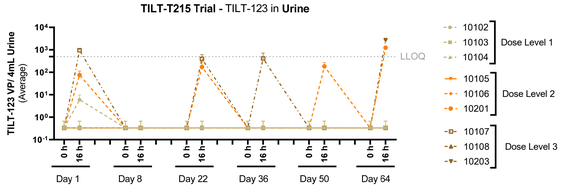
- Treatment with TILT-123 leads to the development of neutralizing antibodies, as expected;
- Less than 50% of evaluated patients had detectable neutralizing antibodies at baseline.

B.



- Shedding of TILT-123 virus genomes in saliva was observed in one patient from dose level 2 and one patient in dose level 3.

C.



- Shedding of TILT-123 virus genomes in urine is increasingly common in response to higher TILT-123 doses;
- TILT-123 is not found pre-dose during treatment.

Figure 6 – Neutralizing antibodies in patients (A), kinetics of virus distribution in Saliva (B) and Urine (C). Data cut-off 30th of August 2022. *The results have not been monitored audited or verified by the quality assurance. LLOQ – Lower Limit of Quantification <500 VP / respective matrix volume. Average values of samples where no TILT-123 was detected (=0) were set to 0.333333 in order to be visibly represented in the log scales of B. and C. graphs.*

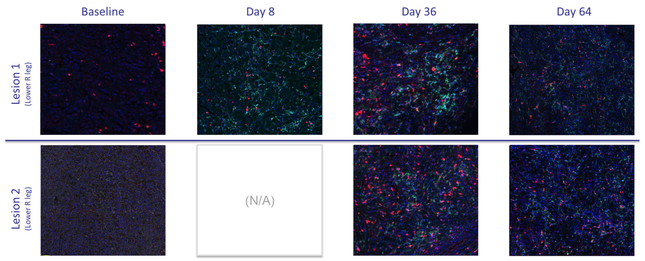


Figure 7 –Example of immune cell infiltration in a tumor from a patient treated in the TILT-T215 demonstrated by multiplex immunohistochemistry. Data cut-off 30th of August 2022. Upper row shows an uninjected tumor, lower row shows an injected tumor *The results have not been monitored audited or verified by the quality assurance.*

- Baseline tumors samples showed lack of T-cell infiltration, indicating that tumors were immune deserted;
- Treatment with TILT-123 induced the intratumoral trafficking of CD8+ T cells;
- In addition, virus treatment increased the presence of CD8+PD-1+ and CD8+Ki67+ T-cells;
- T-cell infiltration was present in non-injected lesion (upper row) and TILT-123-injected lesion (lower row).

Conclusions

- So far, phase I studies indicate that TILT-123 is safe in humans in dose levels 1-3, both as monotherapy and in combination with TIL therapy;
- The safety profile seen in the abovementioned dose levels, is consistent with other virus-based immunotherapies, with the most common AE being fever in both trials;
- Treatment with TILT-123 enabled anti-tumor response as monotherapy. When coupled with another immunotherapy, such as TIL Therapy, it can lead to deepened responses;
- Responses can be observed in lesions in which TILT-123 was not injected;
- Shedding of TILT-123 genomes in saliva and urine samples were detected in some patients treated in the TILT-T215 trial, as expected, however, the presence of functional virus remains to be determined;
- Administration of TILT-123 can enrich the tumor microenvironment with T-cells in melanoma patients from TILT-T215.

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