SITC 2024 – ABSTRACT 672

The tumor-activated IL-12 prodrug WTX-330 expanded and activated tumor infiltrating lymphocytes and caused tumor regression in patients with refractory solid tumors: Interim data from an ongoing Phase 1 study

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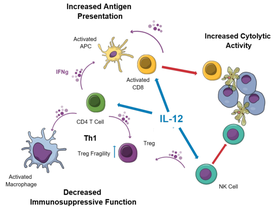
INTRODUCTION

WTX-330 is an engineered prodrug that harnesses the potent, proinflammatory activities of IL-12 to treat challenging cancers



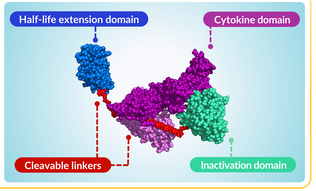
As shown above, the antitumor effects of interleukin-12 (IL-12) result from stimulation of both innate and adaptive immune cells. IL-12 and/or IFNγ, a key effector cytokine induced by IL-12, are known to drive the following activities:

- Induction of T helper type 1 (Th1) cell differentiation
- Activation of cytotoxic natural killer (NK) and T cells
- Reprogramming of immunosuppressive tumor-associated macrophages (TAMs) and regulatory T cells (Tregs)
- Increased antigen presentation by APCs and by tumor cells

The pleiotropic effects of IL-12 may therefore be particularly useful for treating tumors with acquired resistance to standard of care immunotherapies, or for stimulating immune responses in tumors that have not naturally generated antitumor immunity.

Unfortunately, systemic administration of recombinant human IL-12 (rhIL-12) caused severe toxicities (including death) that precluded its clinical development. To improve the therapeutic index for IL-12, Werewolf engineered an INDUKINE™ molecule (WTX-330) to be systemically administered but preferentially activated by dysregulated proteases in the tumor microenvironment, releasing a fully potent, wild type cytokine at the desired site of action. WTX-330 is comprised of:

- **Cytokine domain** – wild type IL-12 heterodimer with native potency and established biology (not a mutein)
- **Inactivation domain** – blocks IL-12 receptor activation when the prodrug is intact to reduce systemic IL-12 toxicity
- **Half-life extension domain** – extends the dosing interval and maximizes tumor prodrug exposure
- **Cleavable linkers** – novel protease substrates optimized for tumor selectivity; WTX-330 cleavage in tumors releases active IL-12



WTX-330x2101 FIRST-IN-HUMAN TRIAL

As of October 7, 2024, twenty-five patients have received at least one dose of WTX-330

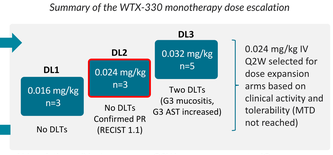
Part 1. Monotherapy dose escalation (n=11)

- Patients with relapsed/refractory solid tumors
- Bayesian logistic regression model study design
- Weight-based dose administered IV Q2W (28-day cycles)

Part 2. Dose expansion (n=14)

- **Arm A (basket):** Patients with IO-sensitive solid tumors who were previously treated with a SOC ICI regimen and who demonstrate 1° or 2° resistance to immunotherapy
- **Arm B (basket):** Patients with tumors for which ICI therapy is neither indicated nor approved (IO-naïve)

Summary of the WTX-330 monotherapy dose escalation



Abbreviations: IV – intravenous, Q2W – every two weeks, IO – immunotherapy, ICI – immune checkpoint inhibitor, SOC – standard of care, DL – dose level, PR – partial response, DLT – dose limiting toxicity

PATIENT DEMOGRAPHICS

First-in-human patient population was heterogeneous and heavily pretreated

Characteristic, n (%)		Dose Escalation (n=11)	Expansion Arm A (n=5)	Expansion Arm B (N=9)	Total (N=25)
Age (years)	Mean (SD)	63.1 (9.4)	60.6 (11.6)	68.2 (7.9)	64.4 (9.4)
	Median	61.0	58.0	70.0	64.0
Sex	Female	5 (45.5%)	2 (40.0%)	5 (55.6%)	12 (48.0%)
	Male	6 (54.5%)	3 (60.0%)	4 (44.4%)	13 (52.0%)
Race	Black/African-American	1 (9.1%)	0 (0.0%)	1 (11.1%)	2 (8.0%)
	White	8 (72.7%)	5 (100.0%)	8 (88.9%)	21 (84.0%)
	Unknown	2 (18.2%)	0 (0.0%)	0 (0.0%)	2 (8.0%)
ECOG PS	0	5 (45.5%)	1 (20.0%)	2 (22.2%)	8 (32.0%)
	1	6 (54.5%)	4 (80.0%)	7 (77.8%)	17 (68.0%)
Tumor type	Colorectal (MSS)	3 (27.3%)	0 (0.0%)	6 (66.7%)	9 (36.0%)
	Melanoma	1 (9.1%)	3 (60.0%)	0 (0.0%)	4 (16.0%)
	PDAC	0 (0.0%)	0 (0.0%)	2 (22.2%)	3 (12.0%)
	NSCLC	0 (0.0%)	2 (40.0%)	0 (0.0%)	2 (8.0%)
	Cholangiocarcinoma	1 (9.1%)	0 (0.0%)	0 (0.0%)	1 (4.0%)
	Endometrial (MSS)	1 (9.1%)	0 (0.0%)	0 (0.0%)	1 (4.0%)
	Urothelial (bladder)	1 (9.1%)	0 (0.0%)	0 (0.0%)	1 (4.0%)
	Other	3 (27.3%)	0 (0.0%)	1 (11.0%)	4 (16.0%)
Prior lines of systemic therapy (non-adjuvant)	0	1 (9.1%)	1 (20.0%)	1 (11.1%)	3 (12.0%)
	1	1 (9.1%)	2 (40.0%)	0 (0.0%)	3 (12.0%)
	2	2 (18.2%)	0 (0.0%)	1 (11.1%)	3 (12.0%)
	3	2 (18.2%)	0 (0.0%)	3 (33.3%)	5 (20.0%)
	≥4	5 (45.5%)	2 (40.0%)	4 (44.4%)	11 (44.0%)
Prior lines of immunotherapy*	0	3 (27.3%)	0 (0.0%)	8 (88.9%)	11 (44.0%)
	1	7 (63.6%)	1 (20.0%)	1 (11.1%)	9 (36.0%)
	2	1 (9.1%)	2 (40.0%)	0 (0.0%)	3 (12.0%)
	3	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
	≥4	0 (0.0%)	2 (40.0%)	0 (0.0%)	2 (8.0%)

Dataset includes all patients treated with at least one dose of WTX-330 by October 7, 2024 (safety evaluable set)

*Lines of immunotherapy include IO agents used alone or in combination with each other or chemotherapy, targeted therapy, etc., for advanced/metastatic disease

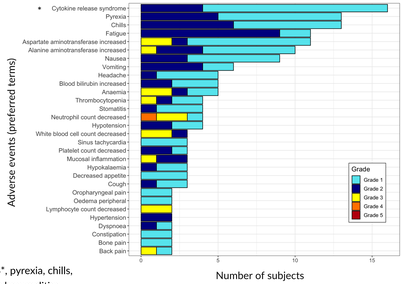
Abbreviations:
SD – standard deviation,
ECOG – Eastern Cooperative Oncology Group,
PS – performance status,
MSS – microsatellite stable,
PDAC – pancreatic ductal adenocarcinoma,
NSCLC – non-small cell lung cancer,
IO – immunotherapy

RELATED TREATMENT-EMERGENT ADVERSE EVENTS (TEAEs)

Related TEAEs were primarily mild to moderate at clinically active doses

Plot shows the frequency of related TEAEs occurring in more than one patient

The data reflect all 25 patients treated with at least one dose of WTX-330 at three dose levels (0.016, 0.024, and 0.032 mg/kg IV Q2W) in the dose escalation and at 0.024 mg/kg IV Q2W in the dose expansion



*Cytokine release syndrome (CRS) was graded by ASTCT scale (Lee DW, et al. *Biol Blood Marrow Transplant* 2019;25(4):625–38)

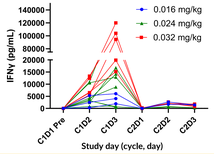
#Leonard JP, et al. *Blood* 1997;90(7): 2541–2548

Key safety findings:

- Most frequent related TEAEs were CRS*, pyrexia, chills, fatigue and elevated liver function test abnormalities
- Notable related TEAEs occurring in only one subject (i.e., not shown) included a G4 lymphopenia, a G3 gastritis, a G3 febrile neutropenia, a G3 drug-induced liver injury, a G3 acute kidney injury, and a G3 activated partial thromboplastin time prolonged
- Gr3 and Gr4 related TEAEs were all manageable and/or reversible with holding/delaying WTX-330 therapy
- CRS and related toxicities improved after the first dose
- Toxicities were typical for systemic IL-12 therapy but less severe (even at 0.032 mg/kg) than rhIL-12 given at its MTD (i.e., in phase 2 study when no test dose was administered)#
- Expansions opened at 0.024 mg/kg because two DLTs were observed at 0.032 mg/kg (Gr3 mucositis, Gr3 AST increase)
- No MTD identified during the dose escalation

PERIPHERAL IFNγ LEVELS CORRELATED WITH CRS-LIKE TOXICITIES

IFNγ "tachyphylaxis" likely accounts for decreased CRS-like toxicities after the first dose of WTX-330



Analysis of plasma IFNγ levels showed:

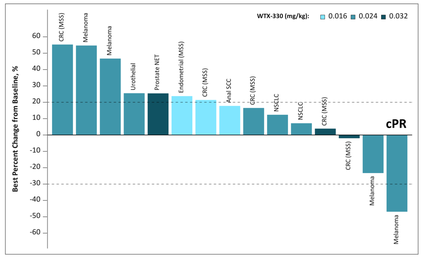
- Dose-dependent induction of IFNγ with the first dose (e.g., C1D2-3)
- Decreased IFNγ with the third dose (e.g., C2D2-3)

Implications:

- Elevated IFNγ with the first dose likely accounts for CRS-like AEs
- Decreased IFNγ with the third dose correlates with improved tolerability
- Decreased IFNγ levels from peripheral IL-12 exposure could potentially be leveraged to improve WTX-330 tolerability and maximize tumor IL-12 exposure

CLINICAL RESPONSES TO WTX-330 MONOTHERAPY

One patient had a confirmed PR and an additional seven patients had stable target lesions*



Abbreviations
CRC – colorectal cancer
MSS – microsatellite stable
NET – neuroendocrine tumor
SCC – squamous cell cancer
NSCLC – non-small cell lung cancer
cPR – confirmed partial response
SOC – standard of care

*Note that response data for a 74-year-old man with pancreatic ductal adenocarcinoma were ascertained but not entered in the database at the time of the October 7 database snapshot and therefore do not appear in the waterfall plot

Patients who demonstrated clinical benefit:

- 76-year-old woman with diffuse melanoma in-transit metastases who progressed during adjuvant pembrolizumab achieved a confirmed PR (RECIST 1.1)
- 77-year-old woman with melanoma who discontinued ipilimumab and nivolumab due to toxicity had a 24% target lesion decrease
- 50-year-old man with MSS CRC who progressed on seven prior lines of therapy including investigational immunotherapies had stable disease for 24 weeks
- 61-year-old woman with MSS CRC who progressed on SOC chemotherapy combined with bevacizumab had stable disease for 16 weeks
- 74-year-old man with pancreatic ductal adenocarcinoma who progressed on SOC chemotherapy and radiation had no growth of the target lesion and no increase in the non-target lesion on the first restaging scan at 8 weeks (ongoing)*

TIMELINE OF RESPONSE IN MELANOMA PATIENT WITH A CONFIRMED PR (RECIST 1.1)

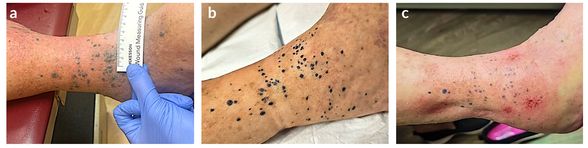
Patient responded to WTX-330 after progressing on adjuvant pembrolizumab

March 2024 (pretreatment; a, d)
Patient was progressing at melanoma in-transit metastases of the right lower extremity and had a melanomatous ulcer. Initiated WTX-330 >2 months after discontinuing pembrolizumab

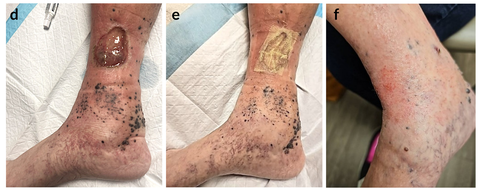
May 2024 (on-treatment; b, e)
After three doses of WTX-330 (0.024 mg/kg IV), many nodules had flattened and/or regressed. Target lesion (cluster of skin nodules) decreased by 47%. Punch biopsies showed no tumor

Sept. 2024 (post-treatment; c, f)
Twelve weeks after discontinuing WTX-330, an ongoing response was noted at a subset of lesions (i.e., with no additional therapy). The patient's melanomatous ulcer had healed

Lateral right lower extremity



Medial right lower extremity



RECIST evaluation

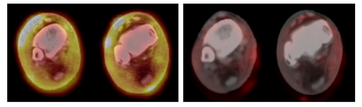
8 weeks: unconfirmed PR based on decreased TL and no increase in size of NTLs

16 weeks: Confirmed PR based on ongoing TL response, complete resolution of one NTL and no increase of a second NTL. Response was ongoing, but patient discontinued treatment due to a related anemia

Abbreviations: RLE – right lower extremity, TL – target lesion, NTL – non-target lesion

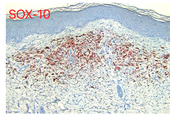
January 2024 (pretreatment):
PET-CT showed progression of RLE melanoma in-transit metastases

May 2024: After three doses of WTX-330, PET-CT showed markedly decreased tumor metabolic activity



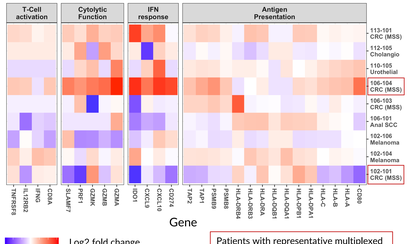
Shown are two different transverse sections of RLE at each timepoint

May 2024: Punch biopsy of pigmented RLE lesion was negative for SOX-10 (melanoma marker) and notable only for melanophages, extracellular melanin deposits and debris



NANOSTRING ANALYSIS OF TUMOR BIOPSIES

WTX-330 increased expression of genes associated with T and NK cell activation and antigen presentation



Patients with representative multiplexed immunofluorescence data (next panel)

Abbreviations: NK – natural killer, IFN – interferon, CRC – colorectal cancer, MSS – microsatellite stable, Cholangio – cholangiocarcinoma, SCC – squamous cell cancer

Key findings from comparison of baseline vs. on-treatment tumor biopsies in nine patients:

- Evidence of pleiotropic IL-12 activity after treatment, including in MSS CRC (note that biopsies for patients 113-101 and 106-104 were taken from liver metastases)
- Increased expression of *IFNG*, *IL12RB2*, *CD8A*, *GRZMB* and *PRF1*, likely reflecting T cell activation and/or enhanced cytolytic function
- Increased expression of *CXCL9*, *CXCL10* and *CD274*, consistent with an IFNγ response
- Upregulation of genes involved with antigen processing and presentation

MULTIPLEXED IMMUNOFLUORESCENCE ANALYSIS OF PAIRED TUMOR BIOPSIES

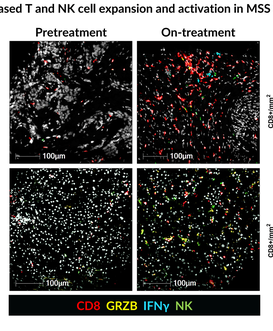
WTX-330 increased T and NK cell expansion and activation in MSS CRC

102-101 MSS CRC
Retroperitoneal lymph node

Overall response: Stable disease (24 weeks)

106-104 MSS CRC
Liver metastasis

Overall response: Progressive disease



Abbreviations: MSS – microsatellite stable, CRC – colorectal cancer, scrn – screening/baseline, C1D22 – cycle 1 day 22 (i.e., after two doses), GrzB – granzyme B, IFNγ – interferon gamma, NK – natural killer

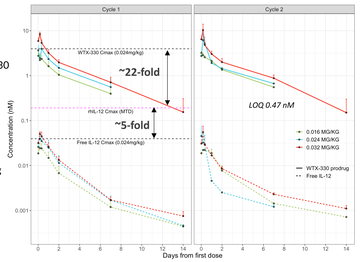
PRELIMINARY WTX-330 PHARMACOKINETIC PROFILE

PK data account for the improved therapeutic index of WTX-330 compared to recombinant human IL-12 (rhIL-12)

Key findings of PK analysis*:

- WTX-330 dosed at 0.024 mg/kg IV Q2W has a **~22-fold higher C_{max} than rhIL-12** dosed at its MTD (500 ng/kg)#, suggestive of effective blockade of peripheral IL-12 activity
- Peak free IL-12 exposure after 0.024 mg/kg WTX-330 is **~5-fold lower than rhIL-12** dosed at its MTD
- Across all dose levels, free IL-12 levels are very low (<1.6% of prodrug exposure)
- WTX-330 PK is approximately dose-proportional from 0.016 to 0.032 mg/kg
- PK exposure generally preserved upon repeat dosing

PK profiles for WTX-330 and free IL-12 compared to rhIL-12 C_{max} when dosed at its MTD (mean ± SEM)



	Parameter Mean (CV%)	0.016 mg/kg (N=3)	0.024 mg/kg (N=3)	0.032 mg/kg (N=5)
WTX-330	Cmax (μg/mL)	0.28 (42%)	0.43 (69%)	0.86 (21%)
	AUC(0-14d)^	1.02 (45%)	1.21 (71%)	1.73 (43%)
Free IL-12	Cmax (pg/mL)	1434.6 (48%)	2297.9 (62%)	2901.8 (30%)
	AUC(0-14d)^	3350.4 (53%)	4941.4 (65%)	5901.5 (38%)

*Preliminary PK data as of June 21, 2024
#Atkins MB, et al. *Clinical Cancer Res* 1997; 3(3):409–17
^units=μg*d/mL; ^units=pg*d/mL

SUMMARY and CONCLUSIONS

- **WTX-330 is the first systemically administered IL-12 therapy with monotherapy clinical activity and a generally tolerable safety profile**
- Second clinical program validating the INDUKINE design for delivery of toxic immune payloads with improved tolerability and clinical benefit
- WTX-330 delivered **22-fold more IL-12 molecules** than rhIL-12 therapy at its MTD, but with lower active IL-12 in plasma
- Related TEAEs were as expected for IL-12 therapy and primarily mild to moderate; severe AEs were manageable, reversible
- Antitumor activity was demonstrated by a confirmed RECIST PR and target lesion shrinkage in two melanoma patients and stable disease for 16 and 24 weeks in two MSS CRC patients and for 8 weeks in a PDAC patient
- NanoString data showed clear evidence of pleiotropic IL-12 activity in the TME
- Tumor biopsies from four patients with MSS CRC showed immune activation, including in liver metastases
- Phase 1/2 dose- and regimen-finding study to optimize WTX-330 exposure in the TME and explore clinical activity in selected indications expected to begin enrolling 1H2025

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