Published OnlineFirst February 4, 2021; DOI: 10.1158/1078-0432.CCR-20-3435

Safety, Antitumor Activity, and T-cell Responses in a Dose-Ranging Phase I Trial of the Oncolytic Peptide LTX-315 in Patients with Solid Tumors

James Spicer[1], Aurélien Marabelle[2], Jean-Francois Baurain[3], Nina Louise Jebsen[4,5], Dag Erik Jøssang[5], Ahmad Awada[6], Rebecca Kristeleit[7], Delphine Loirat[8], George Lazaridis[1], Christiane Jungels[6], Paal Brunsvig[9], Berit Nicolaisen[10], Andrew Saunders[10], Hamina Patel[10], Jérôme Galon[11], Fabienne Hermitte[12], Ketil André Camilio[10], Brynjar Mauseth[10], Vibeke Sundvold[10], Baldur Sveinbjørnsson[10,13], and Øystein Rekdal[10,13]

ABSTRACT

Purpose: LTX-315 is a first-in-class, 9-mer membranolytic peptide that has shown potent immunomodulatory properties in preclinical models. We conducted a phase I dose-escalating study of intratumoral LTX-315 administration in patients with advanced solid tumors.

Patients and Methods: Thirty-nine patients were enrolled, receiving LTX-315 injections into accessible tumors. The primary objective was to assess the safety and tolerability of this approach, with antitumor and immunomodulatory activity as secondary objectives. Tumor biopsies were collected at baseline and posttreatment for analysis of immunologic parameters.

Results: The most common treatment-related grade 1–2 adverse events were vascular disorders including transient hypotension (18 patients, 46%), flushing (11 patients, 28%), and injection site reactions in 38% of patients. The most common grade 3 LTX-315–related toxicities were hypersensitivity or anaphylaxis (4 patients, 10%). Analysis of immune endpoints in serial biopsies indicated that LTX-315 induces necrosis and $CD8^+$ T-cell infiltration into the tumor microenvironment. Sequencing of the T-cell receptor repertoire in peripheral blood identified significant expansion of T-cell clones after treatment, of which 49% were present in available tumor biopsies after treatment, suggesting that they were tumor associated. Substantial volume reduction (≥30%) of injected tumors occurred in 29% of the patients, and 86% (12/14 biopsies) had an increase in intralesional $CD8^+$ T cells posttreatment. No partial responses by immune-related response criteria were seen, but evidence of abscopal effect was demonstrated following treatment with LTX-315.

Conclusion: LTX-315 has an acceptable safety profile, is clinically active, induces changes in the tumor microenvironment and contributes to immune-mediated anticancer activity.

Introduction

Local intratumoral immunotherapy of cancer aims to induce local and systemic immune responses via direct injection of immunostimulatory agents. This should induce tumor cell lysis, followed by the release of tumor-derived antigens and subsequent activation of tumor-specific effector T cells. Other advantages of direct intratumoral therapy include enhanced efficacy and bioavailability of the agent at the local injection site, limited systemic exposure and off-target toxicities, as well as efficient use of drug dosage (1–3).

The oncolytic peptide LTX-315 is a 9-mer peptide designed by structure–activity relationship studies from the host defense peptide lactoferricin (4, 5). It has demonstrated the ability to kill human cancer cells of diverse origin (4, 6). LTX-315 exerts its activity through a membranolytic effect and is equally active against both drug-resistant and drug-sensitive cancer cells. Selectivity for cancer cells results from the cationic amino acid side chains of LTX-315 interacting with the abundant anionic membrane components present at higher levels on cancer cells than in normal cells (7–9).

The oncolytic effect of LTX-315 involves perturbation of both plasma and mitochondrial membrane integrity with subsequent release of danger-associated molecular pattern molecules (DAMP) and tumor antigens. When injected locally into tumors established in immunocompetent mice, LTX-315 induces complete regression and specific anticancer immune responses with subsequent long-term protective immunity (6, 10, 11). Treatment with LTX-315 in these models results in an increase of $CD8^+$ tumor-infiltrating T cells (TIL), and reprogramming of the tumor microenvironment including decreases in the local immunosuppressive regulatory T-cell and myeloid-derived suppressor cell populations (6, 10, 12, 13). In an experimental rat sarcoma model, LTX-315 induced an abscopal effect as demonstrated by regression of distal nontreated lesions, associated with significant infiltration of $CD8^+$ T cells in both treated and non-injected lesions (11).

[1]King's College London, Guy's Hospital, United Kingdom. [2]DITEP, INSERM U1015 & CIC1428, Université Paris Saclay, Gustave Roussy, France. [3]Cliniques Universitaires Saint-Luc, Louvain, Belgium. [4]Centre for Cancer Biomarkers, University of Bergen, Bergen, Norway. [5]Haukeland University Hospital, Bergen, Norway. [6]Institut Jules Bordet, Université Libre de Bruxelles, Brussels, Belgium. [7]University College London Hospital, London, United Kingdom. [8]Institut Curie, Paris, France. [9]Oslo University Hospital, Oslo, Norway. [10]Lytix Biopharma, Oslo, Norway. [11]INSERM Laboratory of Integrative Cancer Immunology, Paris, France. [12]HalioDX, Marseille, France. [13]Department of Medical Biology, Arctic University of Norway, Tromsø, Norway.

Note: Supplementary data for this article are available at Clinical Cancer Research Online (http://clincancerres.aacrjournals.org/).

Trial registration: ClinicalTrials.gov NCT01986426.

Corresponding Author: James Spicer, School Cancer & Pharmacological Sciences, King's College London, 3rd Floor Bermondsey Wing, London SE1 9RT, United Kingdom. Phone: 44-20-7188-7188; E-mail: james.spicer@kcl.ac.uk

Clin Cancer Res 2021;XX:XX–XX

doi: 10.1158/1078-0432.CCR-20-3435

Published OnlineFirst February 4, 2021; DOI: 10.1158/1078-0432.CCR-20-3435

Translational Relevance

A vital mechanistic step underlying the activity of immune-mediated cancer therapy is effective exposure of danger-associated molecular pattern molecules and tumor antigens. LTX-315 is a membranolytic peptide that induces immunogenic cell death. It has demonstrated a marked impact on the tumor microenvironment in preclinical studies. In this phase I trial, we show that intratumoral injection of LTX-315 is well tolerated, has clinical activity including the induction of abscopal responses, and leads to intratumoral effects including an increase of infiltrating $CD8^+$ T cells.

We have conducted a phase I dose-escalating study to assess the safety, tolerability, MTD, pharmacodynamics, and antitumor activity of single-agent intratumorally administered LTX-315 in patients with advanced solid tumors and at least one transdermally accessible lesion.

Patients and Methods

Study design

The study was an open-label multicenter study of LTX-315 with multiple intralesional injections in patients with solid tumors for whom alternative treatment options were not available or suitable (NCT01986426). The primary objective of the study was to assess the safety and tolerability of intratumoral administration of different LTX-315 doses and concentrations. Patients with any solid tumor were eligible for recruitment into the study. As secondary objectives, the antitumor activity by immune-related response criteria (irRC; ref. 14), and pharmacokinetic profile of LTX-315 were evaluated. Blood samples and biopsies of injected tumor were analyzed for pharmacodynamic endpoints.

LTX-315

Manufacturing of LTX-315 was carried out at Bachem in accordance with Good Manufacturing Practice. LTX-315 is a powder for injection formulated by reconstitution in 0.9% saline solution and is administered by intratumoral injection either under direct vision or with ultrasound guidance. In the first-in-man study with LTX-315 (15), 2 patients experienced hypotension following first injection with LTX-315 doses ≥8 mg. This limited the dose of LTX-315 per injection to 7 mg in this study. The total dose of LTX-315 to be administered was determined by dose-escalation cohort. Two to 7 mg of LTX-315 was administered per injection at concentrations of 10 or 20 mg/mL. Three treatment schedules were tested sequentially (**Fig. 1A–C**; Supplementary Table S1A). To break local immune tolerance and build an effective and sustainable systemic immune response, frequent and multiple doses of LTX-315 were administrated during the first weeks of therapy followed by maintenance with less frequent administrations. In the initial schedule (Arm A), LTX-315 was administered to a single lesion on days 1, 2, and 3 during the first week, and thereafter once weekly for a total of 6 weeks. The maintenance phase included one injection per day every 2 weeks for 20 weeks, or until disease progression. A second and third lesion could be treated, and these were injected sequentially, exactly as for lesion 1, in weeks 7 to 12, and then weeks 13 to 18, respectively. Only the last injected lesion was treated in the maintenance phase. This treatment schedule (Arm A) included one injection twice daily (3-hour interval) on each dosing day in the first three cohorts (2, 3, and 4 mg per injection, respectively). Because no grade 2–4 adverse events (AE) were observed after two injections per day (2 + 2 mg = 4 mg, 3 + 3 mg = 6 mg, and 4 + 4 mg = 8 mg), the following cohorts 4–7 included one injection at each dosing day instead of two with 4 mg day 1 and either 5, 6, or 7 mg per injection the following days (Supplementary Table S1A). To allow treatment of multiple lesions per patient, the schedule in Arm B involved either one injection into multiple lesions in parallel on the same dosing day, at days 1, 2, and 3 during the first week, followed by one injection to each lesion in week 2, week 4, and then every 3 weeks until week 49 or progressive disease (Arm B^*), or injection of multiple lesions on the same dosing day, at days 1, 2, 8, 9, 15, and 16 (Arm B^{**}; **Fig. 1A–C**). In the latter schedule, multiple injections per lesion were included per dosing day to compensate for the reduced number of dosing days, and these were separated by 5-minute intervals and injected radially in a clockwise manner. The number of injections per lesion was amended to be dependent upon the lesion size. Arm B^{**} evolved from Arm A and B^* to address emerging hypersensitivity concerns with extended exposure to LTX-315. A maximum of 12 injections were given per dosing day.

Patients

Patients evaluable for antitumor response received at least one injection of LTX-315 and had at least one postdosing tumor evaluation. Eligible patients were ages over 18 years; had unresectable, advanced, or metastatic disease (any solid tumor type); and had received all appropriate available standard-of-care therapies, or were not eligible or suitable for such treatment. All patients had at least one lesion accessible for injection (cutaneous, subcutaneous, oral, or lymph node) which was between 1 and 9 cm in longest diameter, and another noninjected target lesion that was measurable by CT. All patients were required to have Eastern Cooperative Oncology Group performance status of 0 or 1, normal cardiac function, and baseline blood laboratory parameters indicating adequate bone marrow, kidney, and liver function. Patients with a history of systemic autoimmune disease requiring anti-inflammatory therapy or immunosuppressive therapy within the last 3 months prior to study drug administration, incomplete recovery from AEs due to prior therapies, and those with active or unstable cerebral metastases were excluded from the study. Ethical approval for this phase I study was obtained from the Regional Committee of Medical Research Ethics, South-Eastern-Norway (no. 2013/1650), and from the UK NHS Health Research Authority (no. 13/SC/0467). Written informed consent was obtained from patients prior to treatment and the study was conducted in compliance with Good Clinical Practice guidelines after review and approval by appropriate Research Ethics Committees.

Tumor biopsies

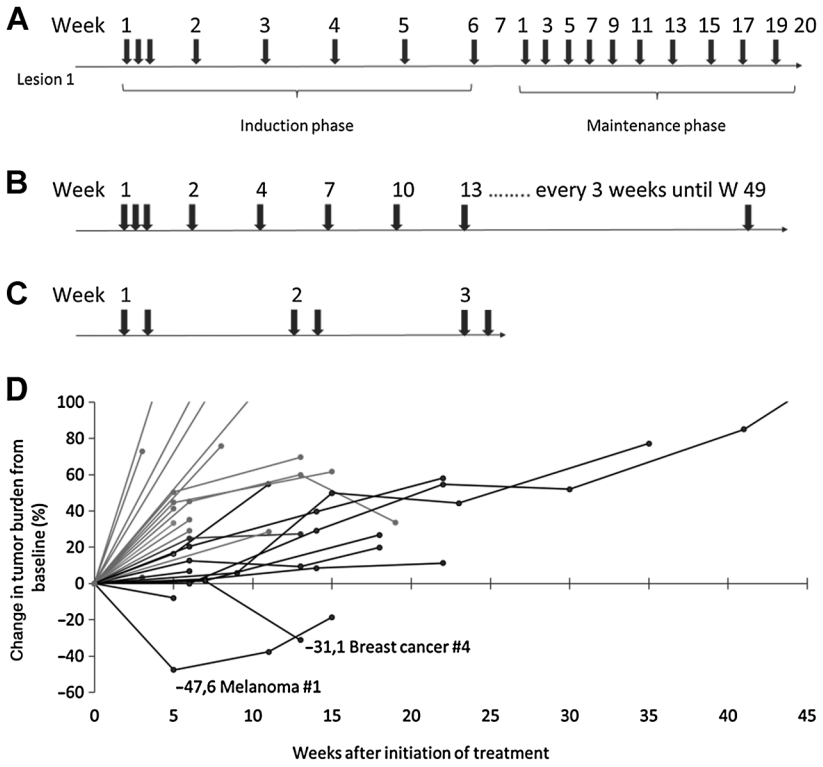
One to three core needle biopsies of the tumor were taken before the first treatment and at week 4 or 7 (contingent on protocol version and arm). The biopsies were embedded in paraffin and stained with hematoxylin and eosin for quality assessment and evaluation of necrosis, followed by IHC staining for tumor microenvironment parameters.

Assessments and endpoints

Safety outcomes

Treatment-emergent AEs (TEAE) were defined as all AEs occurring on or after the first dose of study medication up to 30 days after the last dose. Treatment-related (TR) AEs, deemed by the investigator to

Published OnlineFirst February 4, 2021; DOI: 10.1158/1078-0432.CCR-20-3435



Arm A
Single-lesion injection
Dose 2-7 mg/injection
1 injection per lesion bidaily or daily

Arm B*
Multiple lesions injected concurrently
Dose 3 or 4 mg/injection
1 injection per lesion daily

Arm B**
Multiple lesions injected concurrently
Dose 3, 4 or 5 mg/injection
Number of injections dependent on lesion size

Figure 1.
Treatment schedule and antitumor activity of LTX-315. **A,** LTX-315 was injected in a single lesion on 3 consecutive days and subsequently on 1 dosing day weekly (induction phase). If the patient had another lesion available for injection, a second lesion was injected at week 7 and followed the same treatment schedule as the first lesion. Up to three lesions were treated per patient with this schedule (Arm A). Treatment was terminated with a maintenance phase of 1 dosing day every second week for a total of 20 weeks or to progressive disease. The starting dose for cohort 1 was 2 mg twice per day (4 mg total daily dose) and was escalated to 3 and 4 mg in cohorts 2 and 3, respectively. In the following cohorts in Arm A, LTX-315 was injected once daily, with 4 mg on first dosing day followed by 5, 6, or 7 mg in the subsequent cohorts. **B,** The treatment schedule was amended during the trial to include injections in multiple lesions on the same dosing day (Arm B*) at days 1, 2, and 3 during the first week, followed by one injection to each lesion in weeks 2, 4, and then every 3 weeks until week 49 or progressive disease. **C,** The second part of Arm B (Arm B**) included injection of multiple lesions on the same dosing day, at days 1, 2, 8, 9, 15, and 16. LTX-315 dose was 3, 4, or 5 mg per injection, and the volume (30% of the tumor volume) and number of injections to each lesion was dependent upon the lesion size. **D,** Spider plot showing tumor growth or shrinkage from baseline. The black lines show patients that experienced stable disease assessed by irRC as best response. The best responding patients, melanoma patient #1 and breast cancer patient #4, are indicated.

be related to LTX-315 were tabulated. AEs were summarized by frequency and percentage of patients and categorized by MedDRA version 16.1 system organ class and preferred term unless otherwise indicated and graded according to NCI Common Terminology Criteria for Adverse Events (version 4.0). Serious AEs (SAE), TEAEs leading to treatment discontinuation, and death were also assessed.

Antitumor activity

Overall response was calculated on the basis of the scheduled full tumor assessments by CT/MRI of noninjected lesions. Tumor responses were assessed on the basis of irRC (14). The baseline scan was performed within 28 days prior to the first treatment. In addition to the overall tumor response, change in size was also calculated for individual lesions. A new clinical response criterion has previously been designed to address the unique needs of intratumoral immunotherapy. Intratumoral RECIST (itRECIST) provides guidelines for assessing responses in injected and noninjected lesions as treatment evolves (16). However, these guidelines were not available during the course of the study described herein and therefore not used as an analytic tool in this study.

Pharmacodynamic analyses

IHC. All tumor biopsies were subjected to IHC to assess CD8 expression. Staining was performed on slides using the Roche Ventana Benchmark XT autostainer. After antigen retrieval, staining was performed using CD8 antibodies (M7103, clone C8/144B, or HD-FG-000019) and detected with secondary antibody using Ultraview Universal DAB detection kit. Image acquisition was performed using the Hamamatsu Nanozoomer XR scanner. HalioDx Digital Pathology Platform was used for quantification of CD8 density in the tumor area.

Biopsies from a subset of patients were evaluated by Immunoscore analysis that included staining with anti-CD3 (HD-FG-000013) and anti-CD8 (HD-FG-000019) antibodies on consecutive slides along with digital quantification of T-cell infiltrate (HalioDx).

T-cell receptor repertoire analysis. T-cell receptor (TCR) beta chain CDR3 regions were sequenced by ImmunoSeq (Adaptive Biotechnologies), with primers annealing to V and J segments, resulting in amplification of rearranged VDJ segments from each cell. Clonality values were obtained through the ImmunoSeq Analyzer software (www.adaptivebiotech.com/immunoseq/analyzer). Differential abundance analysis was assessed to identify clones that were significantly

Published OnlineFirst February 4, 2021; DOI: 10.1158/1078-0432.CCR-20-3435

expanded or contracted from baseline to posttreatment timepoints. Significantly expanded clones were identified in peripheral blood and then compared with those in pretreatment and posttreatment tumor samples to determine expansion of tumor-associated T-cell clones.

Gene expression analysis. Identification of upregulated key immune genes involved in tumor regression posttreatment was carried out by hierarchical clustering of Immunosign21 gene signature (HalioDx) by means of a predefined set of genes, including those relating to effector T cells, Th type 1 (Th1) cells, chemokine, and cytokine function (17).

Pharmacokinetics

Plasma was collected before LTX-315 injection and at 1 minute, 5 minutes, 30 minutes, 60 minutes, and 2 hours after dose. Actual sampling time was used to calculate pharmacokinetic parameters. The concentrations of LTX-315 in human plasma samples were measured using LC/MS-MS (API5500 Mass Spectrometer). The samples were analyzed over the calibration range of 2.00–500 ng/mL. Pharmacokinetic data parameters were derived using Phoenix WinNonlin 8.1 (Certara).

Statistical analysis

This was a phase I study with variable number of patients per cohort. No statistical justification has been applied to the number of patients required. In general, statistical analyses were descriptive. Data summaries included frequency counts and percentages for categorical measures and *n*, mean, SD, median, minimum, and maximum values for continuous measurements. All confidence intervals (CI) were two sided. Baseline assessment is the last assessment within 28 days before the first dose of study treatment. Baseline could be the same day as first treatment administration.

Results

Patients

Thirty-nine patients with solid tumors were treated with LTX-315 (**Table 1**) and evaluated for safety. The median age was 60 years (range, 30–80 years). Fifteen patients (38%) were male and 24 patients (62%) were female. Median duration of disease since diagnosis was 60 months (range, 13–234 months).

Table 1. Summary of patient characteristics and previous ICI treatment.

Tumor ($n = 39$)	*N*	Patients with prior ICI treatment
Melanoma	10	10 (100%)
Breast	8	
Head and neck	7	1 (14%)
Sarcoma	6	
Gastrointestinal	3	3 (100%)
Desmoid	2	
Pancreas	1	
Primary vaginal cancer	1	
Carcinoma of unknown primary	1	
Age (median, range)	60 (30–80)	
Male	38%	
Female	62%	
Median time since diagnosis (months)	60.3	

Safety

Thirty-nine patients received at least one dose of LTX-315 and were eligible for analysis of the primary safety endpoint. Aggregated TRAEs reported during the study are presented in **Table 2**, and toxicity data according to arm and cohort is shown in Supplementary Table S1B and S1C. The most common LTX-315–related toxicities were vascular disorders including grade 1–2 hypotension (18 patients, 46%), flushing (11 patients, 28%), and injection site reactions including pruritus (6 patients, 15%), rash (6 patients, 15%), paresthesia (4 patients, 10%), and local pain (2 patients, 5%). Hypotension occurred as an acute effect immediately after intratumoral injection, and in almost all cases was an isolated finding unassociated with features of hypersensitivity. SAEs related to LTX-315 were uncommon and occurred in 4 patients (10%). One patient experienced a grade 4 toxicity of hypersensitivity related to LTX-315 treatment. Clinically significant hypersensitivity reactions were seen following ≥10 weeks of multiple 5 to 7 mg doses of LTX-315 (in 3/14 patients on treatment for this length of time), and when this did occur it was an acute event immediately following an intratumoral injection. Treatment was discontinued in each case following a hypersensitivity reaction. No deaths related to treatment occurred. The treatment schedule was amended to include implementation of prophylactic therapy for hypersensitivity with the use of antihistamines and a leukotriene receptor antagonist, together with a shorter treatment duration of 3 weeks. Following the introduction of these mitigating steps there were no further discontinuations due to hypersensitivity events. Nine out of 39 patients (23%) experienced grade ≥3 events related to LTX-315. Some patients experienced grade ≥3 events in more than one category. All toxicities were manageable and reversible.

Antitumor activity

We assessed antitumor activity using irRC in 27 evaluable patients in all treatment arms, by considering the total tumor burden excluding the injected lesion (**Fig. 1D**). A patient with melanoma had a 48% reduction in tumor burden from baseline, close to the threshold for partial response criteria of 50% regression. Another patient with a progressing retromammary desmoid tumor experienced progressive symptom relief and long-term disease stabilization (>2.5 years) after 16 weeks of treatment, indicating clinical benefit. Prior treatment with IFNα resulted in slowing of tumor growth but treatment was discontinued because of pneumonitis. We believe it is reasonable to attribute the subsequent sustained stable disease to LTX-315 (18). The patient did not have a noninjected lesion evaluable for response and is therefore not included in this analysis. Forty-four percent (12/27) of the patients achieved stable disease, while 56% (15/27) experienced disease progression as best response according to irRC (**Fig. 2A**). Although no objective responses by irRC were seen in patients treated with LTX-315, shrinkage of distant noninjected lesions (abscopal effect) by up to 82% in a single lesion was observed (**Fig. 2A**; Supplementary Table S2). A patient treated in a gluteal leiomyosarcoma lesion had 66% regression of a noninjected lung lesion (**Fig. 2B**). Seven of 24 evaluable patients (29%) had >30% regression in at least one injected lesion.

Pharmacokinetics

Samples from 34 of 39 patients (87%) were available for pharmacokinetic analysis. Transient systemic exposure to LTX-315 was detectable in all but 2 of 34 patients despite the intratumoral route of injection, and was highly variable. Across the cohorts, T_{max} ranged

Published OnlineFirst February 4, 2021; DOI: 10.1158/1078-0432.CCR-20-3435

Table 2. Summary of drug-related TRAEs occurring in at least 10% of patients or grade ≥3 in each arm.

	Arm A ($N = 23$)		Arm B ($N = 16$)		Arm A + Arm B ($N = 39$)	
	Overall	Grade ≥3	Overall	Grade ≥3	Overall	Grade ≥3
Hypotension	15 (65.2%)		3 (18.8%)	1 (6.3%)	18 (46.1%)	1 (2.5%)
Flushing	8 (34.8%)	1 (4.3%)	3 (18.8%)		11 (28.2%)	1 (2.5%)
Pruritus	4 (17.4%)		2 (12.5%)		6 (15.4%)	
Rash	3 (13.0%)		3 (18.8%)		6 (15.4%)	
Musculoskeletal and connective tissue disorders	4 (17.4%)				4 (10.2%)	
Fatigue	4 (17.4%)				4 (10.2%)	
Hypersensitivity	4 (17.4%)	1 (4.3%)			4 (10.2%)	1 (2.5%)
Injection site paresthesia	4 (17.4%)				4 (10.2%)	
Paresthesia	4 (17.4%)				4 (10.2%)	
Anaphylactic reaction	3 (13.0%)	3 (13.0%)			3 (7.7%)	3 (7.7%)
Diarrhea	3 (13.0%)				3 (7.7%)	
Tachycardia					3 (7.7%)	
Hypertension			2 (12.5%)	2 (12.5%)	2 (5.1%)	2 (5.1%)
Rash maculopapular			2 (12.5%)			
Local pain		1 (4.3%)	2 (12.5%)		3 (7.7%)	1 (2.5%)
Syncope		1 (4.3%)				1 (2.5%)
Sepsis		1 (4.3%)				1 (2.5%)

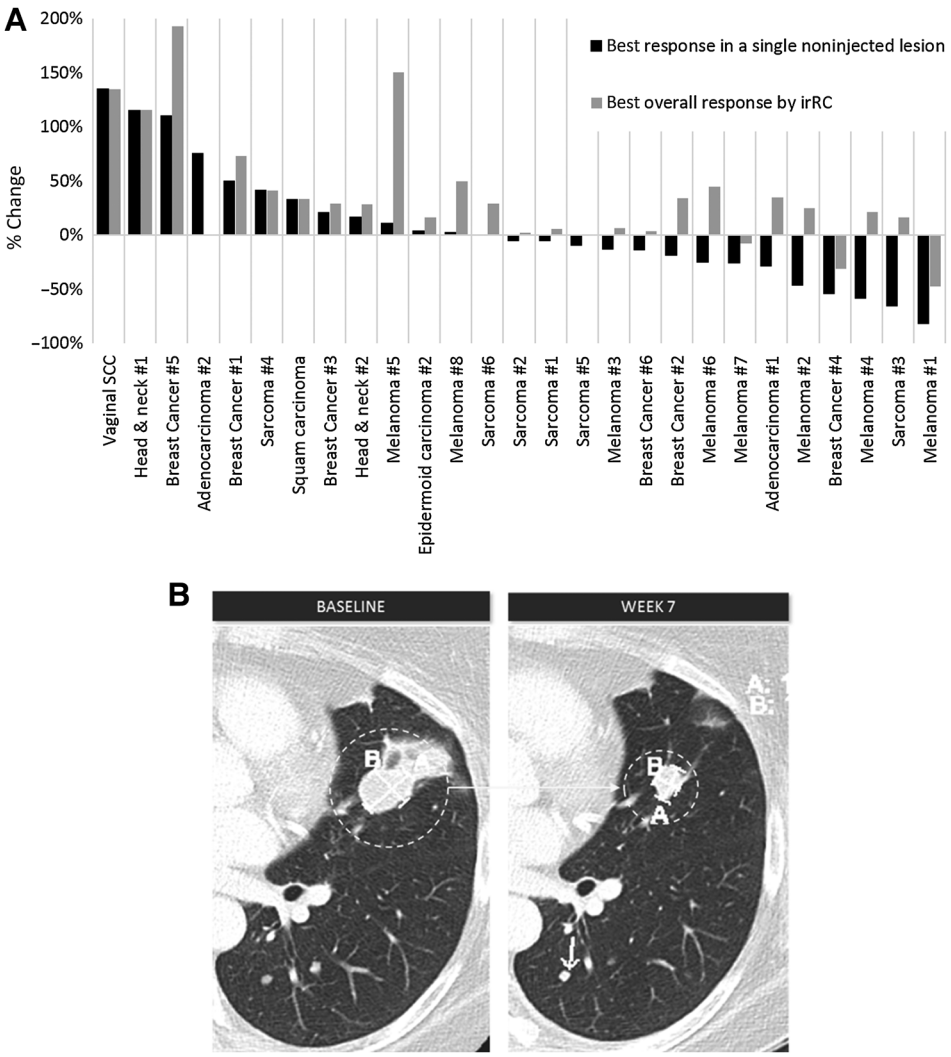


Figure 2.
LTX-315 induces abscopal effects. **A,** Best response at any timepoint after LTX-315 treatment in a single noninjected lesion (black bar) and overall response according to irRC (gray bar) in all patients treated with LTX-315 monotherapy that had CT scans at baseline and after treatment. **B,** Abscopal effect in lung metastatic disease of a patient with recurrent metastatic leiomyosarcoma (sarcoma #3). The patient was enrolled in Arm A and received LTX-315 in the gluteal muscle lesion. Seven weeks after initiation of treatment, one lung lesion had decreased by 64% (product of the two longest perpendicular diameters) from baseline.

Published OnlineFirst February 4, 2021; DOI: 10.1158/1078-0432.CCR-20-3435

Table 3. Pharmacokinetics of LTX-315 in human plasma.

Arm	LTX-315 dose (mg/injection)	*N*	C_{max} (ng/mL)	AUC hour*ng/mL	T_{max} (hour)
A	2	3	24 ± 26	6	0.08
	3	3	6 ± 1	NR	0.03
	4	3	95 ± 104	28	0.02
	5	3	481 ± 720	67 ± 61	0.02
	6	5	427 ± 432	49 ± 40	0.03
	7	4	389 ± 402	617 ± 33	0.26
B*	3	3	59 ± 26	22 ± 19	0.25
	4	2	7[a]	2	0.03
B**	3	4	214 ± 260	155 ± 142	0.68
	4	2	21[a]	7	0.32
	5	2	889 ± 1060	212	0.79

Note: The different arms including distinct treatment schedules and LTX-315 doses per injection are shown. C_{max} is the maximum (or peak) serum concentration of LTX-315 achieved after the drug was administrated. AUC is the integral of the concentration–time curve (after a single dose or in steady state). T_{max} is the time to reach C_{max}. NR, not reportable, insufficient timepoints with drug concentration above the detectable threshold.
[a]Systemic exposure to LTX-315 was not detectable in one 1 out of 2 patients.

from 0.02 to 0.79 hours, and C_{max} ranged from 6 to 890 ng/mL (**Table 3**). In most patients, plasma concentration declined rapidly to low or undetectable levels within 1 hour. Inadequate numbers of serial samples were available for an analysis of half-life.

Pharmacodynamics

Analysis of tumor microenvironment including T-cell infiltration in injected tumors

Paired pre- and posttreatment tumor biopsies were available in 14 of 27 (52%) patients. Only patients with a CT scan after treatment were included for correlation of immune response to clinical benefit. The abundance of intratumoral $CD3^+$ TILs increased after LTX-315 in all patients evaluated, irrespective of the level of TILs in the tumor microenvironment before treatment (**Fig. 3A**). Twelve of 14 (86%) of those paired biopsies showed an increase in $CD8^+$ TILs. Quantitative assessment of biopsies from 5 patients is shown (**Fig. 3B**).

Upregulation of key genes involved in immune tumor rejection

To further investigate the effect of LTX-315 on the tumor microenvironment, and on TILs in particular, gene expression analysis was performed in paired tumor material. Available pre- and posttreatment biopsies with most marked increase in TILs were included in an array analysis. The expression of a panel representing 21 pivotal genes (Immunosign® 21; HalioDx) necessary for mounting an efficient adaptive antitumor immune response was assessed. This panel includes genes involved in activation of cellular immunity, as well as markers of key immune cell subsets (especially cytotoxic T cells and Th1 cells), signaling molecules and receptors. Expression signatures showed that the majority of these genes were upregulated posttreatment, indicating that LTX-315 enriches the tumor microenvironment with adaptive immune components (**Fig. 3C**).

Expansion of tumor-associated T-cell clones

Changes in T-cell clonality in blood were studied in a subset of patients. The frequency of each T-cell clone was examined in pre- and posttreatment blood samples. To identify those T-cell clones whose frequencies differed between pretreated and posttreated samples, an algorithm described by DeWitt and colleagues was used (19). T-cell clones with significant clonal expansion were identified and counted (**Fig. 3D**; Supplementary Fig. S1A). In healthy individuals, an average of 16 T-cell clones have been reported as significantly expanding in a 4-week interval (20). The number of significantly expanded T-cell clones in the patients was above the mean number found in healthy individuals in 4 of 6 (67%), indicating that LTX-315 induces a systemic immune response. A median of 49% of the T-cell clones significantly expanding in blood were also detected in the patients' corresponding tumor specimen posttreatment (Supplementary Fig. S1B). In contrast, the median proportion of significantly expanded T-cell clones in blood also represented in the tumor before treatment was only 23%. The median number of T-cell clones that significantly contracted in blood and present in tumor pre- and posttreatment were 17% and 3%, respectively. Taken together, these data suggest that many of the T-cell clones expanding in patient blood posttreatment are likely to be associated with components of the tumor.

Discussion

Intratumoral immunotherapy is emerging as an effective treatment strategy within the immuno-oncology field. Preclinical data support the hypothesis that by addressing the tumor microenvironment and heterogeneity directly in this way, it may be possible to induce efficient local and systemic tumor-specific immune responses (2, 21–24). Talimogene laherparepvec (T-VEC) was an early intratumoral therapy to receive marketing approval, and many other locally administered therapies with diverse mechanisms of inducing systemic anticancer immunity have entered clinical development (25). In this trial, we show that intratumoral immunotherapy with LTX-315 is well tolerated and results in the induction of local and systemic tumor-specific immune responses. The primary objective of this trial was to explore the safety and tolerability of LTX-315. Thirty-nine patients were treated with intratumoral injections of LTX-315. The safety data demonstrate that transient grade 1–2 hypotension and administration site reactions were common but readily reversible. Hypotension was likely a result of LTX-315 binding to the MRGX2 receptor on basophils in the intravascular space. Binding of cationic antimicrobial peptides to the MRGX2 receptor induces release of histamine, which can result in vasodilator effects (26). Systemic hypersensitivity reactions after extended exposure to LTX-315 for 10 weeks or more was the most clinically significant toxicity (**Table 2**). This was effectively mitigated by introducing prophylactic premedication with antihistamine and leukotriene receptor antagonists, which was effective in preventing recurrence after rechallenge, and by limiting the duration of exposure to multiple injections. It appears that prolonged dosing is the key driver for any hypersensitivity-/anaphylaxis-related AE because clinically significant hypersensitivity to LTX-315 was experienced after >10 weeks of treatment. Moreover, a changed LTX-315 posology including a 3-week dosing schedule and maximum 5 mg per injection was well tolerated.

A number of patients experienced significant tumor regression in injected lesions. Substantial volume reduction (≥30%) of injected tumors occurred in 29% of the patients. More importantly, abscopal responses in distant untreated tumor deposits occurred in several patients (**Fig. 2A** and **B**; Supplementary Table S1), consistent with the concept of local tumor lysis giving rise to a systemic anticancer immune response (2, 22). By inducing immunogenic cell death locally in all cancer cells independent of resistance phenotype, LTX-315 can

Published OnlineFirst February 4, 2021; DOI: 10.1158/1078-0432.CCR-20-3435

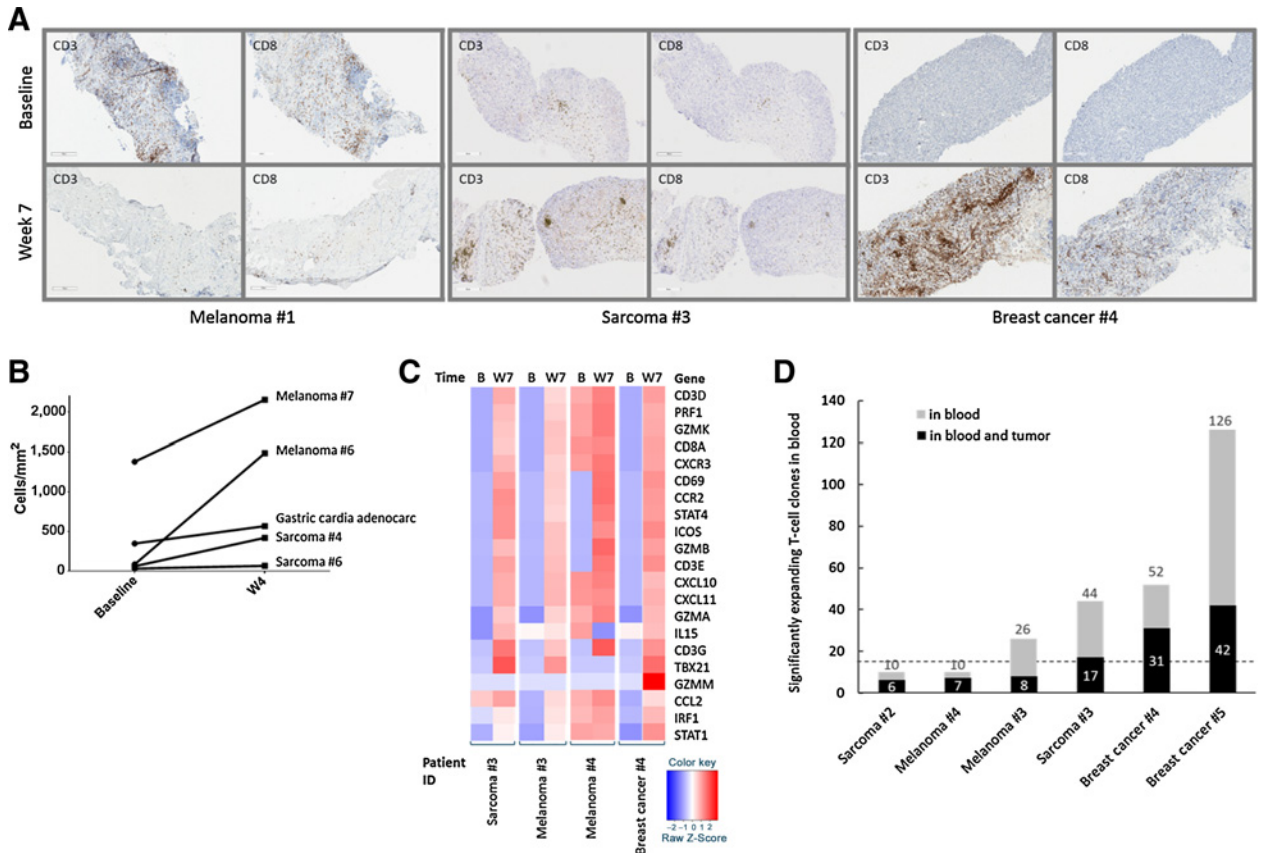


Figure 3.

LTX-315 administration enriches the adaptive immune component of the tumor microenvironment, and induces a polyclonal tumor-associated T-cell response in the periphery. **A,** CD3 and CD8 expression in baseline and posttreatment biopsies from injected lesions in 3 patients enrolled in Arm A. Qualitative assessment of tumor tissue collected from melanoma #1 showed necrosis after treatment, while sarcoma #3 and breast cancer #4 showed an increase in TILs after treatment. **B,** Quantification of $CD3^+$ TILs in injected lesions at baseline and 4 weeks after start of LTX-315 treatment. The number of $CD3^+$ T cells in tumor tissue from patients enrolled in Arm B** is shown. **C,** LTX-315 induces upregulation of key genes involved in immune-mediated tumor regression, including markers of T-cell activation and cytotoxicity. Heatmap showing the expression level of immune response–related transcripts in paired tumor biopsies collected from injected lesions from selected Arm A patients, at baseline (B) and 7 weeks (W7) after start of LTX-315 treatment. Red indicates high expression, blue indicates low expression. **D,** Sequencing of the TCR repertoire in blood before and 7 weeks after treatment for selected patients enrolled in Arm A reveals significant expansion of numerous T-cell clones (shown by the gray). The number of significantly expanding T-cell clones is indicated at top of the bar. The presence of the same T-cell clones in tumor after treatment is shown in the black bar indicating that many of these T-cell clones are tumor associated. The dotted line shows the average number of significantly expanding T-cell clones found in healthy individuals in a 4-week interval.

expose a repertoire of tumor antigens and endogenous danger signals to the immune system, generating a systemic tumor-specific immune response.

An important secondary objective of this trial was to study the immunomodulatory activity of LTX-315. In serial tumor biopsies before and after LTX-315 treatment, we demonstrated an influx of $CD3^+$ and $CD8^+$ TILs to the injected lesions independent of tumor microenvironment status before treatment (**Fig. 3A** and **B**). An increase in $CD8^+$ T cells was observed in 12 of 14 evaluable patients (86%). The presence of TILs, and especially $CD8^+$ T cells, has been recognized as a predictive marker for antitumor immune responses in several indications (27–31). LTX-315 induces release of immunostimulants such as ATP, calreticulin, HMGB1, and cytochrome c from cancer cells (32–34). These DAMPs activate immature dendritic cells and are essential in mounting potent systemic anticancer immune responses, including the infiltration of TILs into the tumor microenvironment (35). As reported previously, this demonstrates that LTX-315 can create local immune responses that correlates with clinical benefit in patients (18), presumably resulting from treatment-induced release of DAMPs and tumor antigens.

Gene expression analysis of tumor biopsies from a subset of patients in this trial has highlighted the ability of LTX-315 to enrich the tumor microenvironment with adaptive immune components in patients with cancer. Substantial changes in injected lesions were demonstrated by a 21-gene mRNA expression panel, demonstrating activation of tumor-specific immune responses. This panel includes genes involved in a Th1 response and Th1 maturation, supporting $CD8^+$ T-cell functions. We observed a significant induction of gene expression involved in cytotoxic T-cell effector function such as perforin and granzymes (36, 37). Transcriptomic analyses have confirmed a strong association between TIL profile and the expression of genes involved in Th1 response and maturation (38). Importantly, Th1 gene signatures have been correlated with good prognosis in several cancers including breast cancer (39) and melanoma (40).

Immunosequencing of TCR is a sensitive, highly accurate technology that quantifies and specifies the clonal distribution of lymphocytes

in biological samples and enables new ways of analyzing treatment responses. Consistent with the LTX-315–mediated modulation of the tumor microenvironment (**Fig. 3C**), we have demonstrated changes in peripheral T-cell clonality posttreatment. Here we show that 4 of 6 patients demonstrate an expansion of circulating T-cell clones after treatment above the level reported in healthy individuals (26–126 significantly expanding T-cell clones; **Fig. 3D**; Supplementary Fig. S1). A larger proportion of these expanding T-cell clones were present in posttreatment tumor tissue compared with pretreatment tumor tissue, indicating that they were tumor associated. Moreover, fewer of the significantly contracted T-cell clones in peripheral blood were present in tumor tissue pre- and posttreatment. Previous studies have shown that clonal T-cell expansion in response to immunotherapy correlates with clinical benefit (41, 42).

Our clinical data also showed a correlation between TILs, gene expression analysis, the number of expanding T-cell clones, and the abscopal effect observed. In patient melanoma #1, who had 82% regression in a noninjected lesion (**Fig. 2A**), the biopsy from the injected lesion was completely necrotic when analyzed by IHC (**Fig. 3A**). Furthermore, nontreated lesions from patient sarcoma #3 and patient breast cancer #4 showed regression of 66% and 54%, respectively (**Fig. 2A**). Treated tumors from these 2 patients exhibited an increase in $CD3^+$ and $CD8^+$ TILs posttreatment (**Fig. 3A**), as well as a transition to a tumor microenvironment richer in adaptive immune components, as analyzed by Immunosign gene expression signature assessment (**Fig. 3C**). Moreover, a significant increase in the number and diversity of expanding T-cell clones in blood was also observed posttreatment (**Fig. 3D**; Supplementary Fig. S1).

Recent developments in immunotherapy approaches have had significant clinical impact in the field of cancer treatment. Antibodies targeting the inhibitory receptors CTLA-4 and PD-1 have shown promising efficacy in several types of cancer (43). However, only subsets of patients experience clinical benefit and preexisting TILs may contribute to clinical response (44). Patients lacking TILs may therefore require therapeutic interventions to prime the T-cell response to enable clinical benefit of checkpoint inhibitor therapy (45). The ability of LTX-315 to increase tumor T-cell infiltration and T-cell clonality therefore positions LTX-315 as a combination partner for other immunotherapies, including immune checkpoint inhibitors. In preclinical tumor models, combination of LTX-315 and an immune checkpoint inhibitor (anti-CTLA-4) demonstrated significant synergy (13). In this phase I trial, we show that intratumoral injection of LTX-315 is well tolerated. The dosing regimen of LTX-315 should be further optimized to position LTX-315 as a therapeutic in combination with checkpoint inhibitors to address an unmet need in a selected group of indications. LTX-315–mediated upregulation of cellular immune components in the tumor microenvironment present a clear rationale for priming a response to checkpoint inhibitors (13). Interestingly, LTX-315 is also being developed as a technology to promote tumor-specific $CD8^+$ T-cell infiltration into tumors, allowing their subsequent harvest, expansion in culture, and infusion as an adoptive T-cell transfer regimen for soft-tissue sarcoma (NCT03725605).

Authors' Disclosures

J. Spicer reports grants from Lytix Biopharma during the conduct of the study, and that he is a co-founder and shareholder of Epsilogen. A. Marabelle reports other from Lytix Biopharma during the conduct of the study, as well as grants, personal fees, and nonfinancial support from Lytix Biopharma outside the submitted work. N.L. Jebsen reports grants from Lytix Biopharma during the conduct of the study. A. Awada reports personal fees from Ipsen, Bayer, Leo Pharma, Merck, Seattle Genetics, Lilly, and Amgen, as well as grants from Roche outside the submitted work. R. Kristeleit reports personal fees from Lytix Biopharma during the conduct of the study; grants and personal fees from Clovis Oncology; personal fees and nonfinancial support from GSK, Roche, AstraZeneca, InCyte, and Basilea; grants from MSD; and personal fees from Pfizer outside the submitted work. J. Galon reports personal fees from HalioDX during the conduct of the study; grants from AstraZeneca, Janssen, and Imcheck Therapeutics; and personal fees from BMS, Merck Serono, Merck MSD, IObiotech, Illumina, Northwest Biotherapeutics, Actelion, Lunaphore, Catalym, Amgen, and Sanofi outside the submitted work. In addition, J. Galon reports a patent for INSERM with royalties paid from HalioDx. F. Hermitte reports a patent for Immunoscore issued to HalioDx. K.A. Camilio reports a patent for immune checkpoint inhibitor combinations issued, a patent for chemotherapeutic combinations of cationic antimicrobial peptides and chemotherapeutics issued, and a patent for methods of generating populations of tumor-infiltrating T cells issued. B. Mauseth is an employee of Lytix Biopharma. V. Sundvold is an employee of Lytix Biopharma. B. Sveinbjørnsson reports a patent for US6,890,902 B2 issued, a patent for US2014/0037657 A1 issued, and a patent for WO 2016/091490 A1 issued, and is employee and shareholder in Lytix Biopharma. Ø. Rekdal reports a patent for US 2019/0038670 issued, a patent for US 2019/0038670 issued, a patent for WO 2016/091490 issued, a patent for JP2016028756A issued, and a patent for AU-2019203199 issued. No disclosures were reported by the other authors.

Authors' Contributions

J. Spicer: Conceptualization, supervision, validation, investigation, writing–original draft, project administration, writing–review and editing. **A. Marabelle:** Supervision, investigation, project administration. **J.-F. Baurain:** Supervision, investigation, project administration. **N.L. Jebsen:** Formal analysis, supervision, investigation, methodology, project administration, writing–review and editing. **D.E. Jøssang:** Formal analysis, supervision, investigation, methodology, project administration. **A. Awada:** Supervision, investigation, project administration. **R. Kristeleit:** Supervision, investigation, project administration. **D. Loirat:** Supervision, investigation, project administration. **G. Lazaridis:** Supervision, investigation, project administration. **C. Jungels:** Supervision, investigation, project administration. **P. Brunsvig:** Supervision, investigation, project administration. **B. Nicolaisen:** Supervision, project administration. **A. Saunders:** Formal analysis, supervision, validation, writing–original draft, project administration. **H. Patel:** Data curation, formal analysis, supervision, validation, writing–original draft, project administration. **J. Galon:** Data curation, formal analysis, validation, methodology. **F. Hermitte:** Data curation, formal analysis, validation, methodology. **K.A. Camilio:** Data curation, validation, visualization, methodology, writing–original draft, writing–review and editing. **B. Mauseth:** Data curation, validation, visualization, writing–original draft, writing–review and editing. **V. Sundvold:** Data curation, formal analysis, validation, writing–original draft, project administration, writing–review and editing. **B. Sveinbjørnsson:** Conceptualization, resources, data curation, formal analysis, methodology, writing–original draft, project administration, writing–review and editing. **Ø. Rekdal:** Conceptualization, resources, writing–original draft, project administration, writing–review and editing.

Acknowledgments

The clinical trial was funded by Lytix Biopharma AS.

The authors particularly thank the patients, their families, and the clinical research teams at all our centers for their contribution to this study. We acknowledge financial support from the United Kingdom Department of Health via the National Institute for Health Research (NIHR) Biomedical Research Centre award to Guy's & St Thomas' NHS Foundation Trust in partnership with King's College London and King's College Hospital NHS Foundation Trust (and NIHR Clinical Research Facility), and to University College London Hospital (UCLH) NHS Foundation Trust. We also acknowledge the funding to the centers of the UK Experimental Cancer Medicine Centre Network at UCLH and King's College London by Cancer Research UK and the UK Departments of Health.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

Received September 11, 2020; revised December 7, 2020; accepted February 2, 2021; published first February 4, 2021.

Published OnlineFirst February 4, 2021; DOI: 10.1158/1078-0432.CCR-20-3435

References

1. Hamid O, Ismail R, Puzanov I. Intratumoral immunotherapy-update 2019. Oncologist 2020;25:e423–e38.
2. Marabelle A, Andtbacka R, Harrington K, Melero I, Leidner R, de Baere T, et al. Starting the fight in the tumor: expert recommendations for the development of human intratumoral immunotherapy (HIT-IT). Ann Oncol 2018;29:2163–74.
3. Hong WX, Haebe S, Lee AS, Westphalen CB, Norton JA, Jiang W, et al. Intratumoral immunotherapy for early-stage solid tumors. Clin Cancer Res 2020;26:3091–9.
4. Haug BE, Camilio KA, Eliassen LT, Stensen W, Svendsen JS, Berg K, et al. Discovery of a 9-mer cationic peptide (LTX-315) as a potential first in class oncolytic peptide. J Med Chem 2016;59:2918–27.
5. Sveinbjornsson B, Camilio KA, Haug BE, Rekdal O. LTX-315: a first-in-class oncolytic peptide that reprograms the tumor microenvironment. Future Med Chem 2017;9:1339–44.
6. Camilio KA, Berge G, Ravuri CS, Rekdal O, Sveinbjornsson B. Complete regression and systemic protective immune responses obtained in B16 melanomas after treatment with LTX-315. Cancer Immunol Immunother 2014;63:601–13.
7. Gaspar D, Veiga AS, Castanho MA. From antimicrobial to anticancer peptides. A review. Front Microbiol 2013;4:294.
8. Riedl S, Zweytick D, Lohner K. Membrane-active host defense peptides–challenges and perspectives for the development of novel anticancer drugs. Chem Phys Lipids 2011;164:766–81.
9. Utsugi T, Schroit AJ, Connor J, Bucana CD, Fidler IJ. Elevated expression of phosphatidylserine in the outer membrane leaflet of human tumor cells and recognition by activated human blood monocytes. Cancer Res 1991;51:3062–6.
10. Liao H-W, Garris C, Pfirschke C, Rickelt S, Arlauckas S, Siwicki M, et al. LTX-315 sequentially promotes lymphocyte-independent and lymphocyte-dependent antitumor effects. Cell Stress 2019;3:348–60.
11. Nestvold J, Wang MY, Camilio KA, Zinocker S, Tjelle TE, Lindberg A, et al. Oncolytic peptide LTX-315 induces an immune-mediated abscopal effect in a rat sarcoma model. Oncoimmunology 2017;6:e1338236.
12. Camilio KA, Wang M-Y, Mauseth B, Waagene S, Kvalheim G, Rekdal Ø, et al. Combining the oncolytic peptide LTX-315 with doxorubicin demonstrates therapeutic potential in a triple-negative breast cancer model. Breast Cancer Res 2019;21:9.
13. Yamazaki T, Pitt JM, Vetizou M, Marabelle A, Flores C, Rekdal O, et al. The oncolytic peptide LTX-315 overcomes resistance of cancers to immunotherapy with CTLA4 checkpoint blockade. Cell Death Differ 2016;23:1004–15.
14. Wolchok JD, Hoos A, O'Day S, Weber JS, Hamid O, Lebbe C, et al. Guidelines for the evaluation of immune therapy activity in solid tumors: immune-related response criteria. Clin Cancer Res 2009;15:7412–20.
15. Brunsvig P. A phase I study with LTX-315, an immunogenic cell death inducer, in patients with transdermally accessible tumors. J Clin Oncol 32: 15s, 2014 (suppl; abstr 3067).
16. Goldmacher GV, Khilnani AD, Andtbacka RHI, Luke JJ, Hodi FS, Marabelle A, et al. Response criteria for intratumoral immunotherapy in solid tumors: itRECIST. J Clin Oncol 2020;38:2667–76.
17. Galon J, Bindea G, Mlecnik B, Angell H, Lagorce C, Todosi AM, et al. Microenvironnement immunitaire et cancer. Med Sci 2014;30:439–44.
18. Jebsen NL, Apelseth TO, Haugland HK, Rekdal O, Patel H, Gjertsen BT, et al. Enhanced T-lymphocyte infiltration in a desmoid tumor of the thoracic wall in a young woman treated with intratumoral injections of the oncolytic peptide LTX-315: a case report. J Med Case Rep 2019;13:177.
19. DeWitt WS, Emerson RO, Lindau P, Vignali M, Snyder TM, Desmarais C, et al. Dynamics of the cytotoxic T cell response to a model of acute viral infection. J Virol 2015;89:4517–26.
20. Rytlewski J, Deng S, Xie T, Davis C, Robins H, Yusko E, et al. Model to improve specificity for identification of clinically-relevant expanded T cells in peripheral blood. PLoS One 2019;14:e0213684.
21. Hammerich L, Bhardwaj N, Kohrt HE, Brody JD. *In situ* vaccination for the treatment of cancer. Immunotherapy 2016;8:315–30.
22. Hammerich L, Binder A, Brody JD. *In situ* vaccination: Cancer immunotherapy both personalized and off-the-shelf. Mol Oncol 2015;9:1966–81.
23. Pierce RH, Campbell JS, Pai SI, Brody JD, Kohrt HE. *In-situ* tumor vaccination: bringing the fight to the tumor. Hum Vaccin Immunother 2015;11:1901–9.
24. Sagiv-Barfi I, Czerwinski DK, Levy S, Alam IS, Mayer AT, Gambhir SS, et al. Eradication of spontaneous malignancy by local immunotherapy. Sci Transl Med 2018;10:eaan4488.
25. Aznar MA, Tinari N, Rullán AJ, Sánchez-Paulete AR, Rodriguez-Ruiz ME, Melero I. Intratumoral delivery of immunotherapy-act locally, think globally. J Immunol 2017;198:31–9.
26. Pundir P, Kulka M. The role of G protein-coupled receptors in mast cell activation by antimicrobial peptides: is there a connection? Immunol Cell Biol 2010;88:632–40.
27. Edlund K, Madjar K, Mattsson JSM, Djureinovic D, Lindskog C, Brunnström H, et al. Prognostic impact of tumor cell programmed death ligand 1 expression and immune cell infiltration in NSCLC. J Thorac Oncol 2019;14:628–40.
28. Galluzzi L, Senovilla L, Zitvogel L, Kroemer G. The secret ally: immunostimulation by anticancer drugs. Nat Rev Drug Discov 2012;11:215–33.
29. Martin de la Fuente L, Westbom-Fremer S, Arildsen NS, Hartman L, Malander S, Kannisto P, et al. PD-1/PD-L1 expression and tumor-infiltrating lymphocytes are prognostically favorable in advanced high-grade serous ovarian carcinoma. Virchows Arch 2020;477:83–91.
30. Trujillo JA, Sweis RF, Bao R, Luke JJ. T cell-inflamed versus non-T cell-inflamed tumors: a conceptual framework for cancer immunotherapy drug development and combination therapy selection. Cancer Immunol Res 2018;6:990–1000.
31. Van Berckelaer C, Rypens C, van Dam P, Pouillon L, Parizel M, Schats KA, et al. Infiltrating stromal immune cells in inflammatory breast cancer are associated with an improved outcome and increased PD-L1 expression. Breast Cancer Res 2019;21:28.
32. Eike LM, Yang N, Rekdal O, Sveinbjornsson B. The oncolytic peptide LTX-315 induces cell death and DAMP release by mitochondria distortion in human melanoma cells. Oncotarget 2015;6:34910–23.
33. Zhou H, Forveille S, Sauvat A, Sica V, Izzo V, Durand S, et al. The oncolytic peptide LTX-315 kills cancer cells through Bax/Bak-regulated mitochondrial membrane permeabilization. Oncotarget 2015;6:26599–614.
34. Zhou H, Forveille S, Sauvat A, Yamazaki T, Senovilla L, Ma Y, et al. The oncolytic peptide LTX-315 triggers immunogenic cell death. Cell Death Dis 2016;7:e2134.
35. Kepp O, Senovilla L, Vitale I, Vacchelli E, Adjemian S, Agostinis P, et al. Consensus guidelines for the detection of immunogenic cell death. Oncoimmunology 2014;3:e955691.
36. Church SE, Galon J. Regulation of CTL infiltration within the tumor microenvironment. In: Kalinski P, editor. Tumor immune microenvironment in cancer progression and cancer therapy. Cham: Springer International Publishing; 2017. p. 33–49.
37. Martínez-Lostao L, Anel A, Pardo J. How do cytotoxic lymphocytes kill cancer cells? Clin Cancer Res 2015;21:5047–56.
38. Galon J, Costes A, Sanchez-Cabo F, Kirilovsky A, Mlecnik B, Lagorce-Pagès C, et al. Type, density, and location of immune cells within human colorectal tumors predict clinical outcome. Science 2006;313:1960–4.
39. Ascierto ML, Kmieciak M, Idowu MO, Manjili R, Zhao Y, Grimes M, et al. A signature of immune function genes associated with recurrence-free survival in breast cancer patients. Breast Cancer Res Treat 2012;131:871–80.
40. Mann GJ, Pupo GM, Campain AE, Carter CD, Schramm S-J, Pianova S, et al. BRAF mutation, NRAS mutation, and the absence of an immune-related expressed gene profile predict poor outcome in patients with stage III melanoma. J Invest Dermatol 2013;133:509–17.
41. Page DB, Yuan J, Redmond D, Wen YH, Durack JC, Emerson R, et al. Deep sequencing of T-cell receptor DNA as a biomarker of clonally expanded TILs in breast cancer after immunotherapy. Cancer Immunol Res 2016;4:835–44.
42. Snyder A, Nathanson T, Funt SA, Ahuja A, Buros Novik J, Hellmann MD, et al. Contribution of systemic and somatic factors to clinical response and resistance to PD-L1 blockade in urothelial cancer: an exploratory multi-omic analysis. PLoS Med 2017;14:e1002309.
43. Darvin P, Toor SM, Sasidharan Nair V, Elkord E. Immune checkpoint inhibitors: recent progress and potential biomarkers. Exp Mol Med 2018;50:1–11.
44. Sharma P, Allison JP. The future of immune checkpoint therapy. Science 2015;348:56–61.
45. Galon J, Bruni D. Approaches to treat immune hot, altered and cold tumours with combination immunotherapies. Nat Rev Drug Discov 2019;18:197–218.

AACR American Association for Cancer Research

Clinical Cancer Research

Safety, Antitumor Activity, and T-cell Responses in a Dose-Ranging Phase I Trial of the Oncolytic Peptide LTX-315 in Patients with Solid Tumors

James Spicer, Aurélien Marabelle, Jean-Francois Baurain, et al.

Clin Cancer Res Published OnlineFirst February 4, 2021.