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Tilsotolimod Exploits the TLR9 Pathway to Promote Antigen Presentation and Type 1 IFN Signaling in Solid Tumors: A Multicenter International Phase I/II Trial (ILLUMINATE-101).

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Tilsotolimod exploits the TLR9 pathway to promote antigen presentation and Type 1 IFN signaling in solid tumors, A Multicenter International Phase I/II trial (ILLUMINATE-101)

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Abstract

Purpose: Tilsotolimod is an investigational synthetic Toll-like receptor 9 (TLR9) agonist that has demonstrated anti-tumor activity in preclinical models. The ILLUMINATE-101 phase 1 study explored the safety, dose, efficacy, and immune effects of intratumoral (it) tilsotolimod monotherapy in multiple solid tumors.

Patients and Methods: Patients with a diagnosis of refractory cancer not amenable to curative therapies received tilsotolimod in doses escalating 8 to 32 mg into a single lesion at weeks 1, 2, 3, 5, 8, and 11. Additional patients with advanced Malignant Melanoma were enrolled into an expansion cohort at the 8 mg dose. Objectives included characterizing the safety, establishing the dose, efficacy, and immunological assessment. Blood samples and tumor biopsies of injected and noninjected lesions were obtained at baseline and 24 hours after treatment for immune analyses.

Results: Thirty-eight and 16 patients were enrolled into the dose escalation and melanoma expansion cohorts, respectively. Deep visceral injections were conducted in 91% of patients. No dose-limiting toxicities (DLT) or grade 4 treatment-related adverse events were observed. Biopsies 24 hours after treatment demonstrated an increased IFN pathway signature and dendritic cell maturation. Immunologic profiling revealed upregulation of IFN-signaling genes and modulation of genes for checkpoint proteins. In the dose escalation cohort, 12 (34%) of 35 evaluable patients achieved a best overall response rate (ORR) of stable disease (SD) whereas 3 (19%) of 16 evaluable patients in the melanoma cohort achieved stable disease.

Conclusion: Overall, tilsotolimod monotherapy was generally well tolerated and induced rapid, robust alterations in the tumor microenvironment.

Statement of translational relevance:

Toll-like receptor 9 (TLR9) agonists demonstrated efficacy in activation of the innate and adaptive immune system in preclinical models. We investigated the activity of tislotolimod, an investigational synthetic Toll-like receptor 9 agonist, in patients with refractory solid tumors after progression on standard-of-care therapies. Our early phase clinical trial established the maximum tolerated dose, safety of this novel new platform drug, and demonstrated early signs of efficacy of tilsotolimod. In addition, translational data revealed activation of the innate and adaptive immune system through activation of type I interferon (IFN) pathway, INFγ and INFα gene upregulation, increase in inflammatory chemokines, activation in myeloid dendritic cells and antigen presentation, and increase in genes for checkpoint and co-stimulatory proteins. The translational results highlight a role for investigating tilsotolimod in combination with checkpoint inhibitors in solid tumors.

Keywords

TLR9; melanoma; intratumoral; sarcoma

Introduction

T cell-targeted cancer immune therapies such as checkpoint inhibitors (CPI) provide durable, systemic anti-tumor responses to some patients, but the effects rarely extend to less immunogenic tumors. Treatments that stimulate the innate immune system and reverse the immunosuppressive tumor microenvironment represent an emerging strategy to promote anti-tumor immunity either alone or in combination with CPIs for patients with immunologically cold tumors. Toll-like receptors (TLR) are expressed by an array of immune cells and enable a non-specific immune response to pathogen-associated molecular patterns, biochemical patterns unique to pathogens (1). They trigger innate immunity by increasing the production of interferon and other cytokines and activating antigen presenting cells, resulting in increased activation of effector T cells. TLR9 is located in endosomal compartments and is predominantly expressed by plasmacytoid dendritic cells (pDC) and B cells, elements of the innate and adaptive immune systems, respectively (2,3). Direct, intratumoral stimulation of TLR9 with synthetic agonists promotes a local anti-tumor response via activation of the innate and ultimately the adaptive immune systems that can

subsequently lead to systemic immunity. This limits systemic immune-related toxicities compared to systemic administration of CPIs (4).

The TLR9 agonist IMO-2125 (tilsotolimod) is a synthetic phosphorothioate oligodeoxynucleotide consisting of two strands that are 3'-3' linked. Intratumoral (i.t.) tilsotolimod demonstrated immune mediated anti-tumor efficacy in murine syngeneic lymphoma and colon carcinoma models by an increase in CD3+T lymphocytes within the tumor microenvironment and an upregulation of selected checkpoint genes including *PD-1, PD-L1, CEACAM1, OX40, OX40L*, *CTLA-4, LAG3*, and *TIM3* (5). Additionally, in preclinical studies, tilsotolimod induced high levels of IFN-alpha from dendritic cells (DC) along with B-cell proliferation and differentiation and significantly augmented cytotoxic T cell responses against tumor antigens leading to regression of injected and distant, non-injected tumors, suggestive of an abscopal effect (6-8). These findings are suggestive of both potential single-agent activity use and in combination with CPIs. In a phase I/II clinical study of i.t. tilsotolimod and ipilimumab or pembrolizumab in patients with advanced melanoma who progressed on or after treatment with a PD-1 inhibitor, both combinations were generally well tolerated (9,10). In this study, evaluable patients who received the 8 mg dose of tilsotolimod combined with ipilimumab (N=49) achieved an overall response rate (ORR) of 22.4% and a disease control rate (DCR) of 71%. Tumor reduction was observed in both the injected and non-injected lesions. Seven of the 11 responses lasted for ≥ 6 months, including an ongoing complete response of nearly 4 years. Tumor biopsies revealed a robust, early activation of a type I interferon response and DC activation in the injected lesion along with CD8+ T cell proliferation in both injected and non-injected tumors (9). This led to a phase III randomized control trial in patients with advanced melanoma (ILLUMINATE-301; ref. 11).

Here we present a phase I/II dose escalation trial of single agent tilsotolimod in patients with refractory solid tumors to determine feasibility, safety, recommended phase II dose (RP2D), pharmacokinetics, clinical activity, immunologic activity, and biomarkers for immunologic assessment. This is one of the first clinical trials to study TLR9 agonists via i.t. injection in solid tumors other than advanced melanoma and to investigate the feasibility and safety of visceral i.t. injections. We hypothesized that treatment with tilsotolimod will modulate the tumor microenvironment in microsatellite stable solid tumors and lead to DC activation, increases in type I IFN, stimulate cytotoxic T-cell responses, upregulate checkpoint genes, and demonstrate an abscopal effect. Herein, we provide mature results from both the dose-escalation and expansion cohorts.

Patients and Methods

Study Design

This open-label, monotherapy, multicenter Phase 1b dose evaluation study (NCT03052205) assessed the safety, pharmacokinetics (PK), preliminary clinical activity, immune effects, and recommended phase 2 dose (RP2D) of i.t. tilsotolimod monotherapy in multiple solid tumor types. The study was run in 2 parts, the dose evaluation/escalation portion, and the dose expansion portion. In the dose escalation phase, patients received i.t. tilsotolimod in doses escalating from 8 mg to 32 mg (8 mg, 16 mg, 23 mg, and 32 mg) into a single

lesion on day 1 of weeks 1, 2, and 3 (3-week cycle), and then on day 1 of week 1 of a 3-week cycle, for up to a maximum of 17 total cycles (Supplementary Fig. S1 and Supplementary Table S1). Cohorts of approximately 8 patients were planned to be enrolled sequentially with dose escalation proceeding unless >2 patients experienced a DLT. An additional eight patients were planned to be enrolled at the RP2D. Patients with advanced melanoma were enrolled into an expansion cohort at the RP2D of 8 mg i.t. tilsotolimod previously established based on another study, ILLUMINATE-204 (NCT02644967), in combination with ipilimumab for metastatic melanoma refractory to PD-1 inhibitor. The study was approved by institutional review boards at each participating center. The study was conducted according to the Declaration of Helsinki and Good Clinical Practices. Written informed consent was obtained prior to enrollment.

Objectives

Dose escalation cohort—The primary objective of the dose escalation cohort was safety. Secondary objectives were to establish a RP2D, assess clinical activity of i.t. tilsotolimod monotherapy, and characterize its pharmacokinetics.

Melanoma expansion cohort—The primary objective of the melanoma dose expansion cohort was investigator-assessed ORR using RECIST v1.1. Secondary objectives were safety, other measures of clinical benefit, and pharmacokinetics.

Exploratory Objectives—Exploratory objectives were similar between the 2 cohorts and included characterizing biomarkers for immunologic assessment and assessing anti-drug antibodies.

Patient Selection

Eligible patients were adults with a histologically or cytologically confirmed diagnosis of metastatic solid tumor malignancies that are refractory to available therapies. Patient were required to have at least one lesion accessible for i.t. injection, an Eastern Cooperative Oncology Group performance status (ECOG PS) of ≤2, and adequate hematologic, renal, and hepatic function. Patients with a diagnosis for which a PD-(L)1/PD-1 inhibitor has been approved must have previously received treatment with one of these therapies. Key exclusion criteria included prior therapy with a TLR agonist, treatment with IFN-α within the previous 6 months, active autoimmune disease requiring disease-modifying therapy, concurrent systemic steroid therapy (>10 mg/day of prednisone or equivalent), and active central nervous system metastases.

Treatment Administration

Tilsotolimod was administered as a series of i.t. injections into a single tumor selected for injection. The injected (primary) tumor for each patient was selected from pathologic draining lymph nodes, deep (visceral) metastases, and superficial or subcutaneous metastases. Deep injected tumors required real-time image-guided (US, CT) delivery utilizing interventional radiology techniques. In the event a full dose could no longer be practically administered into the injected tumor, another tumor could be selected for injection. In the case of complete remission, remaining injections were to be administered

into the tumor bed, except in the case of visceral lesions where remaining injections were to be administered subcutaneously at a location based on the investigator's preference and discretion. Tilsotolimod was thoroughly distributed within the injected tumor while avoiding necrotic areas, using a fanning method to distribute the administered volume throughout the injected lesion. The total injected dose was to remain constant for each patient; however, injection volume could be adjusted dependent on the size of tumor.

Study Procedures

Clinical and laboratory safety assessments were conducted at baseline, weekly during cycle 1, then on day 1 of subsequent cycles. Adverse events were graded using the Common Terminolgy Criteria for Adverse Events (CTCAE) version 4.03. Imaging and efficacy assessments occurred every 6 weeks (dose-escalation cohort) or 9 weeks (melanoma expansion cohort) during the treatment period and then at least every 12 weeks during follow-up and included clinical examination and CT or magnetic resonance imaging of known sites of disease. Tumor response was assessed using RECIST v1.1 and immune related Response Evaluation Criteria in Solid Tumors (irRECIST). Blood samples for plasma preparation and bioanalysis of tilsotolimod concentrations were collected prior to each injection during Cycles 1-3, during the follow-up safety visit, and post-dose at intervals (30 minutes, 1, 2, 3, 4, and 24-48 hours) on day 1 of Cycles 1 and 2.

Core biopsies of the injected (primary) tumor and another distant tumor (if available) were taken at screening, 24 hours after the first dose of i.t. tilsotolimod on Day 1 of Cycle 1, and approximately 6 weeks after the first dose (primary biopsy only). Core biopsies were optional in the melanoma expansion cohort.

Immune analyses included NanoString (NanoString Technologies) and/or flow cytometry of type I interferon (IFN) pathway activation, IFN-γ levels, activation of dendritic cell subsets, and changes in T cell status.

RNA was extracted from core needle biopsies preserved in RNA later using the Qiagen AllPrep Universal Kit (Cat # 80224) according to the manufacturer's instructions. Purity and concentration were assessed using NanoDrop. Gene set scores were generated from the Pan Cancer Immune Panel (NanoString Technologies) and analyzed using the nSolver Advanced Analysis Software.

Statistical Methods

The first part of the study was dose evaluation. Patients were treated in 4 dose escalation cohorts (8 mg, 16 mg, 23 mg, and 32 mg). DLTs and treatment-emergent adverse event (TEAE) were graded based on CTCAE v4.03. For the dose evaluation cohort, results were summarized descriptively. Summary statistics for continuous variables included number of observations, mean, standard deviation (SD), median, minimum, and maximum. Categorical variables were summarized using frequency counts and percentages. Descriptive statistics were provided for all PK parameter values by dose and time. Data from all participating sites were pooled prior to analysis. Baseline was defined as the most recent, non-missing value prior to the date and time of the first dose of study drug. Study population analysis and safety analyses were based on the Safety Set. Summaries of AEs included only TEAEs,

defined as AEs with a start date on or after the date of first dose of study drug or existing AEs which increased in CTCAE grade after the start of study treatment. All other safety analyses included only post-baseline measures. All efficacy analyses were based on the efficacy evaluable set. The best overall response was based on RECIST v1.1 criteria and response assessments of complete response (CR) and partial response (PR) required confirmation by imaging ≥ 4 weeks after the initial documentation of CR or PR. The primary analysis of PK was conducted on the PK Population. A nonlinear power model was used to assess the dose proportionality of tilsotolimod on Day 1 of Cycle 1 for the four dose evaluation cohorts. The melanoma dose expansion portion used a Simon's Optimal Two Stage design with a type I error rate of 0.05 and 80% statistical power to test the hypothesis that the response rate per RECIST v1.1 was ≥ 30%, which is clinically meaningful in this setting.

Data availability

The authors confirm that the data supporting the findings of this study are available within the article and/or its supplementary materials.

Results

Patients and Disease Characteristics

The first patient was enrolled on May 15, 2017, and the last patient completed treatment on July 31, 2019. Fifty-four patients with refractory advanced solid tumors were enrolled, including 38 in the dose-evaluation portion and 16 patients with refractory melanoma patients in the expansion cohort. Deep, visceral lesions were injected with the aid of image guidance in 91% of patients (n=49). All patients successfully received injected tilsotolimod doses to specified lesions. Overall, patients in the dose evaluation portion of the study had a mean age of 56.3 years (range: 20 to 77 years). The majority of the patients were females (25, 65.8%). In the dose-evaluation cohort, the most common cancer types were pancreatic (n=12) and colorectal cancer (n=7). Most (n=35, 92.1%) patients had Stage IV disease, 23 (60.5%) had received prior targeted therapy, and 8 (21.1%) had received prior anti-PD-1 therapy (Table 1). Eight, 10, and 9 patients received the 16, 23, or 32 mg i.t. tilsotolimod dose, respectively, whereas 11 patients received the RP2D of 8 mg (Supplementary Table S1).

Patients in the melanoma expansion cohort, study had a mean age of 61.7 years (range: 18 to 86 years) and nine of the 16 patients (56.3%) were male. All patients had Stage IV disease and had received prior checkpoint inhibitor therapy 13 (81.3%) anti-PD-1, 10 (62.5%) anti-CTLA-4, and 5 (31.25%) as a combination. Most patients (n=10, 62.5%) had elevated LDH, and 4 (25%) had BRAF mutation positive melanoma.

Safety

No treatment-related DLTs were observed. All 54 patients had at least one treatment-emergent adverse event (TEAE) (Table 2). Treatment-related adverse events (TRAE) were mostly ≤ grade 2 with 8 (14.8%) patients having grade 3 TRAEs. The most common TRAEs were pyrexia, fatigue, chills, nausea, and vomiting. Fatigue was the only frequent TRAE to

increase with the dose. Only one patient required dose reduction due to AEs. There were no AEs resulting in death in both the dose evaluation and dose-expansion cohorts. There were no DLTs observed in the four dose escalation cohorts and responses were seen across the different doses levels and hence the recommended phase II (RP2D) was determined to be 8 mg.

Clinical Responses

Dose-evaluation cohort—Thirty-five patients were evaluable for clinical response assessment with a median follow-up of 2.6 months. Twelve (34.3 %) patients achieved a best overall response of stable disease (SD; Table 3). Tumor reduction was observed in both the injected lesion and distant, non-injected lesion; five patients demonstrated a reduction in the longest diameter of either the injected or non-injected lesions (Figure 1a). Noninjected lesions were all visceral lesions and included hepatic, pulmonary, or other intrabdominal masses. The median duration of SD was 5.1 months (minimum 1.5, maximum 12.6), with 1 patient ongoing for more than 1 year (Figure 1b and c). SD was observed in at least two patients at each dose level and in several tumor types, including three of seven patients with soft-tissue sarcoma and four of seven patients with colorectal cancer. There was no correlation between a best overall response of SD and baseline characteristics or any treatment-related AE. Three patients (two colorectal cancer and one sarcoma) achieved SD for more than 10 months after progressing on all lines of standard-of-care therapy (Fig 1b).The two colorectal cancer patients were in the 23 mg cohort and had stable disease for >24 weeks and both developed PD at the end of cycles 15 and 17. The sarcoma patient had uterine leiomyosarcoma and was in the 32 mg cohort and had SD for >24 weeks and during active follow-up visits continued to have SD.

Melanoma expansion cohort—All 16 patients were evaluable for clinical response assessment with a median follow-up of 6.4 months. Similar to the dose evaluation cohort, tumor reduction in either the injected or non-injected lesions occurred in five patients (31.3%) (Figure 1a). In this monotherapy expansion cohort in heavily pretreated population with advanced melanoma, SD was the best overall response (n=3, 18.8%) (Figure 1a). Patients had a median progression-free survival of 2.2 months (95% CI 1.9 - 4.0) and median overall survival (OS) of 8.5 months (95% CI 4.5 - not evaluable: NE).

Pharmacokinetics

Due to the intratumoral route of tilsotolimod administration, variability in drug plasma concentration is expected and influenced by multiple factors. Tilsotolimod was quickly absorbed from the injection site into systemic circulation with a T_{max} of approximately 0.5 hours and a half-life of < 2 hours. There was no quantifiable tilsotolimod 1 week after dosing. Drug exposure was dose proportional for AUC_{inf} and C_{max}. The PK profiles were similar between the cohorts.

Immunologic Profiling of Tumors

Similar to prior pre-clinical of i.t. tilsotolimod, a rapid, robust activation of the type I IFN pathway was consistently observed in biopsies obtained 24 hrs after tilsotolimod i.t. injection compared with baseline levels (Figure 2). Specifically, several IFN-signaling genes

were upregulated including genes that encode transcription factors (*STAT1, STAT2, IRF7*), anti-viral proteins (*Cig5, OAS3, MX1*), cell death/survival factors (*MG1P3, IFI27*), and signaling regulators (*IFIT4, Ly6E*).

Immune profiling of gene families revealed that IFNγ, IFN downstream, and inflammatory chemokines were the most heavily upregulated gene sets in 24 hr biopsies and then returned to near baseline levels 6 weeks after dosing (Figure 3a). Analysis of paired biopsies pre- and 24 hours post-tilsotolimod administration also found that the IFNα gene expression signature was substantially upregulated in many tumor types (Fig 3b). Importantly, all 24 hr biopsies had a highly significant increase in IFNα gene expression signature ($P < 10E-5$).

To examine potential functional effects of elevated IFN signaling, the maturation of type 1 myeloid dendritic cells (mDC1) was assessed by flow cytometry in fresh biopsy samples. Upregulated HLA-ABC expression indicative of mDC1 activation was observed in one out of three available samples [numbers of CD1c-positive cells (mDC1) increased by 4.4-fold], whereas mDC2 maturation was observed in two of three samples [numbers of CD141-positive cells (mDC2) increased by 2.3-fold and 3.3-fold; Fig 3c].

The capacity for robust antigen presentation was also elevated in 24 hr post-tilsotolimod biopsies. *TAP1* and *TAP2*, transporters responsible for MHC peptide loading, were upregulated by 2.4-fold and 2.2-fold, respectively, along with IFN-induced protein with tetratricopeptide repeats 2 (IFIT2) by 6.3-fold (Figure 2)

Genes for checkpoints and co-stimulatory proteins were also modulated 24 hr after tilsotolimod injection. *PD-1, PD-L2, TIM3*, and *LAG3* were all significantly increased, though *CTLA-4* expression was not significantly changed. Additionally, expression of co-stimulatory proteins *CD80* and *CD86* was increased (Supplementary Table S2). Overall, tilsotolimod had a substantial impact on the innate immune activation profile of solid tumors.

Discussion

We have demonstrated feasibility, safety, and tolerability as well as an immunomodulatory effect of a synthetic CpG oligonucleotide injected i.t. in non-MSI-H refractory solid tumors (12). Treatment with tilsotolimod revealed potential preliminary evidence of clinical activity across multiple solid tumors including those traditionally unresponsive to immunotherapy. Of the 45 patients who were evaluable for response, 33.3% had stable disease including some who had rapidly progressed on prior treatment, such as two colorectal cancer patients and one sarcoma patient who achieved SD for more than 10 months.

This was a dose-escalation trial, however, no DLTs were observed and no patient discontinued treatment due to TRAEs). The most common TRAEs (pyrexia, fatigue, nausea, and chills) were similar to side effects previously reported with other CpG agonists (13,14). As no DLTs were observed and efficacy was seen across different doses, the recommended phase II (RP2D) was determined to be 8 mg. This trial also demonstrated a unique finding of decrease in size of not only injected but also non-injected measurable lesions, suggesting an abscopal effect. This was seen previously in a clinical trial investigating the combination

of TLR9 agonist and radiation in low-grade non-Hodgkin's lymphoma, however, we report this finding as monotherapy in solid tumors which suggests CpG agonist as the cause and not only radiation (14) . Immune monitoring analysis showed robust activation of the type I interferon pathway in injected lesions demonstrated by increased IRF7, IFIT1, and IFIT2 gene expression and early increases in type I interferon signaling. Subsequent analysis also demonstrated increase in dendritic cell activation, upregulation of MHC class II, and up-regulation of interferon-alpha signaling, suggesting improved antigen presentation. This was observed across multiple tumor types, and changes were consistent with those observed in a previous phase I/II clinical trial of patients with metastatic melanoma (9). Moreover, immune profiling gene expression from baseline revealed an approximately 2-fold increase in the expression of immune checkpoint gene mRNA, including LAG-3, PD-1, and PD-L2 (12). These findings provided, in part, the rationale for exploring the potential for tilsotolimod to complement CPIs in advanced solid tumors, which is being studied in patients with colorectal cancer in combination with nivolumab and ipilimumab (ILLUMINATE-206) and a phase III trial (ILLUMINATE-301) studying the combination of tilsotolimod and ipilimumab versus ipilimumab in patients with advanced melanoma refractory to PD-1. However, it is important to note that that the ILLUMINATE-301 failed to meet its primary endpoint of ORR. We look forward to reviewing the final publication to evaluate the details of efficacy analysis including OS and correlative biomarkers. It is important to note that the ILLUMINATE-301 enrolled patients with malignant melanoma only and not patients with advanced solid tumors as is the case in our current trial.

In our trial, most of the patients were heavily pretreated and had aggressive malignancies with guarded prognosis such as pancreatic adenocarcinoma, soft tissue sarcoma, and osteosarcoma. These data with single agent intratumoral tilsotolimod administration are promising and support further investigation of use in combination with other immunotherapeutic agents such as CPIs. Although it is important to note the negative phase III trial (ILLUMINATE-301) in patients with malignant melanoma and the median OS of 8.5 months in the melanoma expansion cohort in our current trial indicate no role as single agent and questionable efficacy when combined with CPI in this disease. In patients with colorectal cancer, we look forward to reviewing the results from the ILLUMINATE-206 trial.

In conclusion, tilsotolimod shows clinical activation of the innate and adaptive immune response via rapidly increasing dendritic cell activation, upregulation of MHC-class II, and IFN-alpha (antigen presentation) and induces a 2-fold increase in the expression of immune check point genes including inducing an "abscopal effect" in refractory non-MSI-H solid tumors. These findings provide a rationale for ongoing trials in solid tumors combined with CPI; however, this will need to be revisited after evaluating upcoming data from the ILLUMINATE-301 phase III trial.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

CR	Complete Response
IT	Intratumoral
ORR	Objective Response Rate
PR	Partial Response
SAE	Serious Adverse Event
SD	Stable Disease
TLR9	Toll-like Receptor 9
TEAE	Treatment-emergent Adverse Event

References

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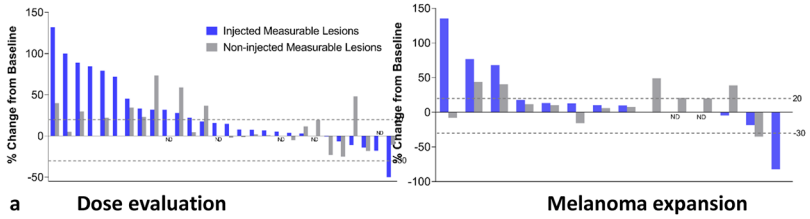


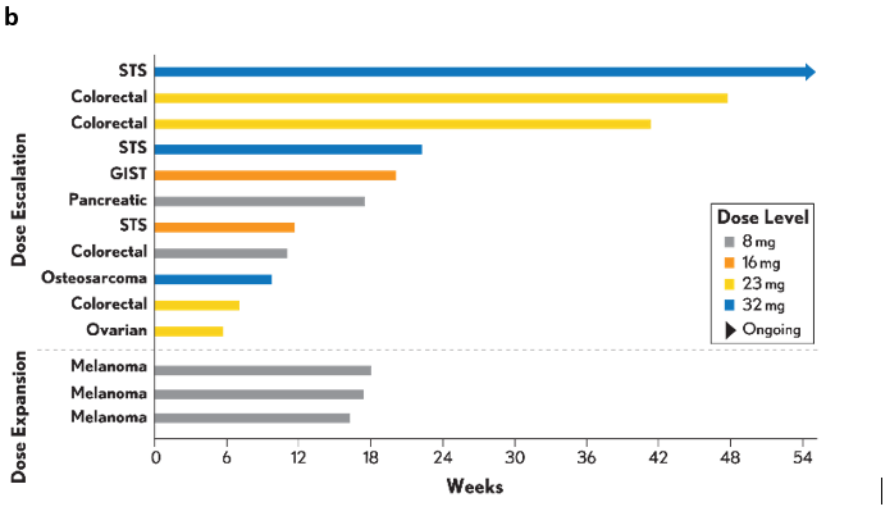
SLDL sum of the longest diameters

ND, not determined

[a] includes patients with at least 1 post-baseline assessment with measurements of all measurable lesions

Tumor regression of injected and noninjected lesions (RECIST v1.1)

Patients	Regression in injected lesion (%)	Regression in noninjected lesion (%)
1	-10.80%	N/A
2	N/A	-2.08
3	N/A	-1.42
4	N/A	-5
5	-0.50	-23.20
6	-6.45	-25
7	-50.0	-10.71
8	-14.8	-18.18
9	-17.94	N/A
10	N/A	-16.60
11	-82.2%	N/A
12	N/A	-10.00



GIST, gastrointestinal stromal tumor; STS, soft tissue sarcoma.

One patient had an assessment of stable disease after discontinuing treatment due to clinical progression and is excluded from the figure.

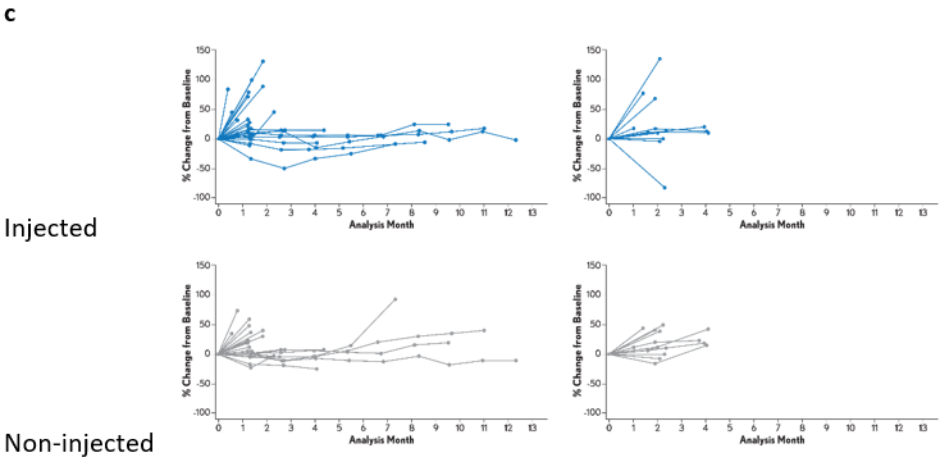


Figure 1:

(Efficacy): **a)** Waterfall plot: Maximum percent reduction from baseline in individual sum of the longest diameters (SLD) of injected and non-injected lesions in patients in the dose evaluation and expansion cohorts; Table of tumor regression of injected and noninjected lesions. **b)** Swimmer plot: duration of patients in the clinical trial; each bar represents one patient. **c)** Spaghetti plot: Individual percent change from baseline in SLD of target lesions

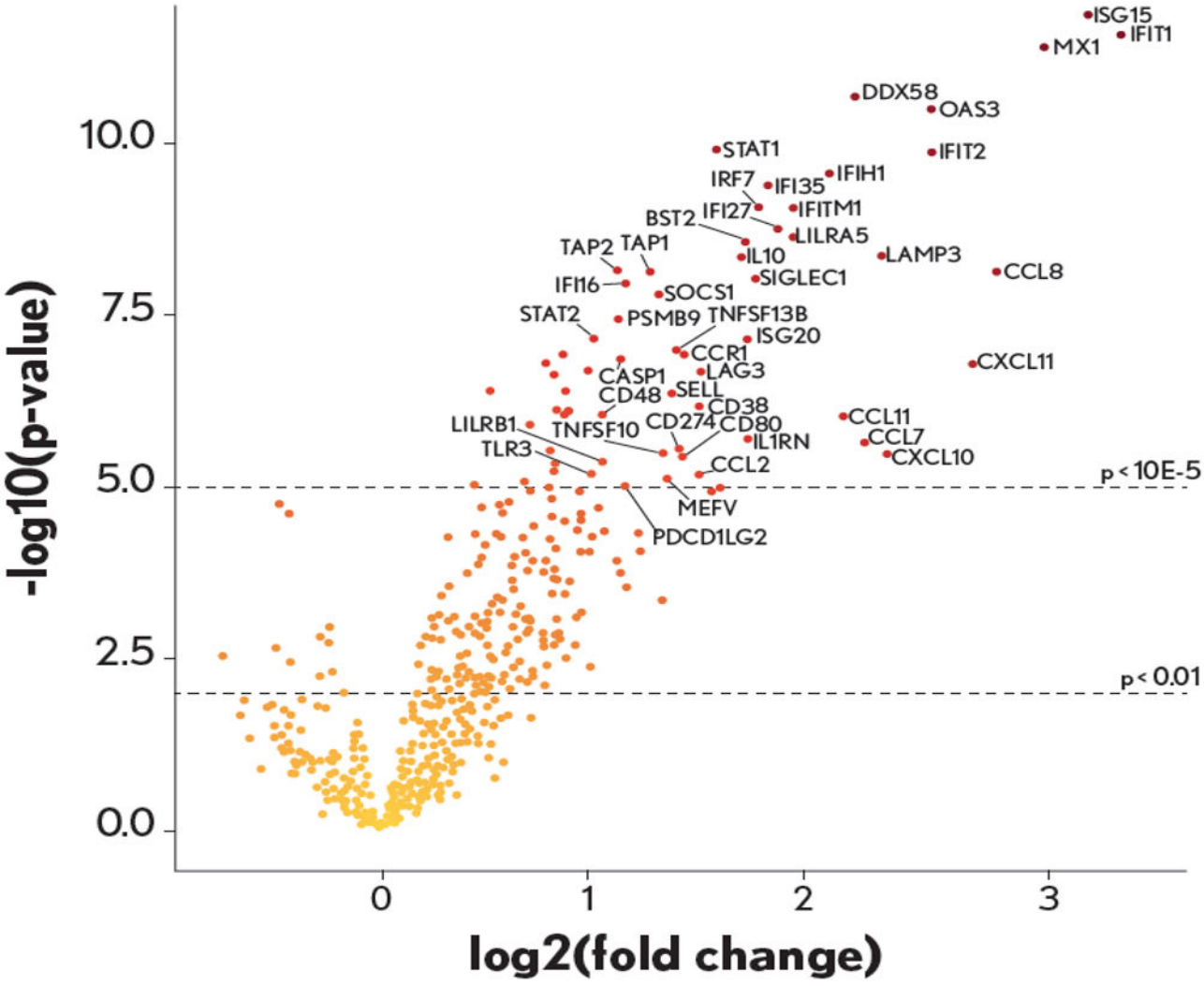


Figure 2:

Immune profiling of tumor biopsies: Volcano plot of RNA extracted from the local injected lesion at 24h after tilsotolimod as compared with baseline. The adjusted *p* value is indicated. Volcano plot showing robust activation of the type 1 IFN pathway in injected lesions demonstrated by increased interferon regulatory factor 7 (IRF7), interferon induced protein with tetratricopeptide repeats 1 (IFIT1), and interferon induced protein with tetratricopeptide repeats 2 (IFIT2) gene expression and early increases in type 1 IFN signaling. Other upregulated IFN-signaling genes include transcription factors (STAT1, STAT2, IRF7), antiviral proteins (Cig5, OAS3, MX1), cell death/survival factors (MG1P3, IF127), and signaling regulators (IFIT4, Ly6E). This highlights robust activation of the innate immunity by tilsotolimod.

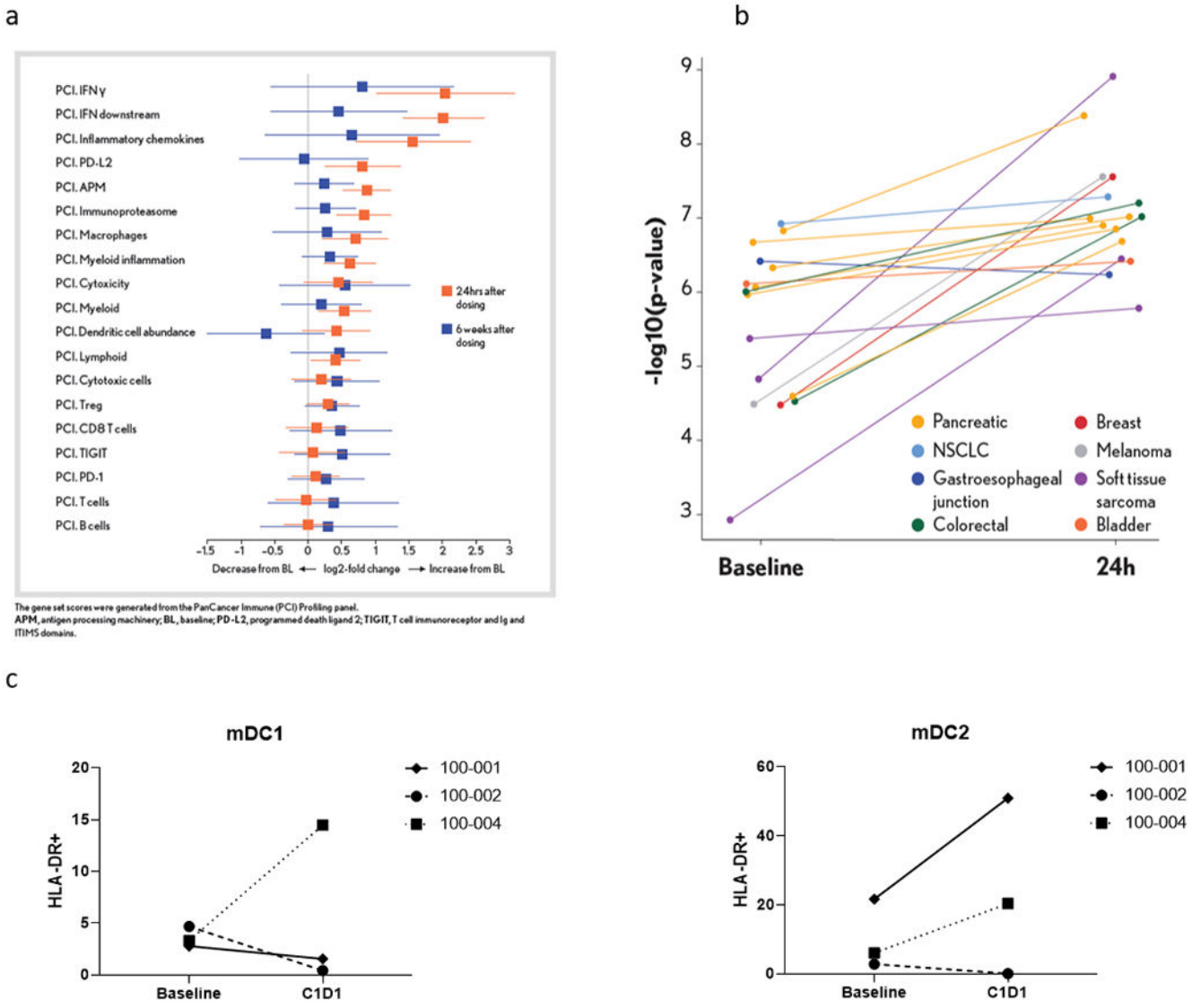


Figure 3:

Rapid alterations in immune signaling pathways: a) Forest plot showing changes in gene families obtained from Nanostring analysis. b) Change in IFN-a levels from RNA extracted from the local injected lesion at 24h after tilsotolimod as compared with baseline. c) Flow cytometry data showing changes in HLA-DR expression in the local injected lesion at 24 hr after tilsotolimod as compared with baseline.

Table 1:

Patients and baseline characteristics

Characteristic N (%)	Dose-evaluation (N = 38)	Melanoma expansion (N = 16)
Gender (F/M)	25,13(65.8,34.2)	7,9(43.8,56.3)
Median age (min, max), years	59.5 (20, 77)	65.0 (18, 86)
ECOG PS 0-1	38 (100)	16 (100)
Ethnicity		
White	35 (92.1%)	16 (100%)
African American/Black	1 (2.6%)	
Asian	1 (2.6%)	
Hispanic	4 (10.5%)	
Other	1 (2.6%)	
Cancer diagnosis		n/a
Pancreatic	12 (31.6)	
Colorectal	7 (18.4)	
Gastro-esophageal	2 (5.3)	
Other	17 (44.7)	
Metastatic disease	35 (92.1)	16 (100)
Elevated LDH	n/a	10 (62.5)
BRAF V600 Mutation	n/a	4 (25.0)
Prior Treatment		
Chemotherapy	34 (89.5)	9 (56.3)
PD-1 inhibitor	8 (21.1)	13 (81.3)
CTLA-4 inhibitor	1 (2.6)	10 (62.5)
Other immunotherapy	6 (15.8)	1 (6.3)
Kinase inhibitor	9 (23.7)	3 (18.8)
Other targeted therapy	14 (36.8)	0
Median Prior Systemic Therapies (min, max)	7.0 (1, 18)	3.0 (2, 10)

Table 2:

TRAEs by Grade

Adverse Event, n (%)	Dose Evaluation (N = 38)	Melanoma Expansion (N = 16)
≥ 1 TEAE	38 (100)	16 (100)
≥ 1 Grade 3/4 TEAE	18 (47)	7 (44)
≥ 1 Grade 3 TRAE [a]	6 (16)	2 (13)
≥ 1 SAE	13 (34)	7 (44)
Most common grade 3/4 TEAEs		
Anemia	3 (8)	1 (6)
Fatigue	2 (5)	0
Sepsis	2 (5)	0
Hyponatremia	2 (5)	0
AST increased	2 (5)	0
Thrombocytopenia	2 (5)	0
Most common TRAEs [b]		
Pyrexia	23 (61)	12 (75)
Fatigue	13 (34)	6 (38)
Chills	13 (34)	3 (19)
Nausea	4 (11)	5 (31)
Vomiting	3 (8)	5 (31)

Abbreviations: AST, Aspartate aminotransferase; SAE, serious adverse event; TEAE, treatment-emergent adverse event; TRAE, treatment-related adverse event.

[a] No grade 4 TRAEs were observed

[b] Most common TRAEs observed in the total population

Table 3:

Best Overall Response

Response	Dose-escalation (N = 35)	Melanoma expansion (N = 15)
Objective Response Rate (%)	0 (0)	0 (0)
Disease Control Rate (%)	12 (34.3)	3 (18.8)
Complete Response (%)	0 (0)	0 (0)
Partial Response (%)	0 (0)	0 (0)
Stable Disease (%)	12 (34.3)	3 (18.8)
Progressive Disease (%)	20 (57.1)	10 (62.5)