Original Article

First-in-human study of LY3039478, an Oral Notch signaling inhibitor in advanced or metastatic cancer
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The results of this Phase I trial demonstrate the safety and preliminary antitumor activity of LY3039478 as a single agent. The clinical pharmacodynamic effect of LY3039478 on Notch targeted genes is considered as a major strength of this study. Overall, this work supports the rational for targeting Notch signalling and further implicates aberrant Notch signalling in tumor physiology.
ABSTRACT

**Background:** Deregulated Notch signaling due to mutation or overexpression of ligands and/or receptors is implicated in various human malignancies. Gamma-secretase inhibitors inhibit Notch signaling by preventing cleavage of transmembrane domain of Notch protein. LY3039478 is a novel, potent Notch inhibitor decreases Notch signaling and its downstream biologic effects. In this first-in-human study, we report the safety, pharmacokinetic profile, pharmacodynamic effects, and antitumor activity of LY3039478 in patients with advanced or metastatic cancer.

**Methods:** This phase I, open-label, multicenter, nonrandomized, and dose-escalation phase study determined and confirmed the recommended phase II dose of LY3039478 (oral dose: 2.5–100 mg, TIW on a 28-day cycle). The primary objectives are to determine (Part A) and confirm (Part B) a recommended phase II dose that may be safely administered to patients with advanced or metastatic cancer, and secondary objectives include evaluation of safety, tolerability, pharmacokinetic (PK) parameters, and preliminary antitumor activity of LY3039478.

**Results:** A total of 110 patients were treated with LY3039478 monotherapy between 31 October 2012 and 15 July 2016. Dose-limiting toxicities were thrombocytopenia, colitis and nausea. Most adverse events were gastrointestinal. The recommended phase 2 dose was 50 mg TIW, because of its better tolerability compared to 75 mg. The pharmacokinetics of LY3039478 appeared dose proportional. Pharmacodynamic data indicate an approximately 80% inhibition of plasma Aβ, and >50% inhibition of Notch-regulated genes hairy and enhancer of split-1, cyclin D1 and Notch-regulated ankyrin repeat at the 45-to 100-mg dose. Clinical activity was observed in patients with breast cancer, leiomyosarcoma, and adenoid cystic carcinoma.

**Conclusion:** Potent inhibition of Notch signaling by LY3039478 was well tolerated at doses associated with target engagement, and demonstrated evidence of clinical activity in heavily
pretreated patients. Further investigation with LY3039478 as monotherapy and in combination with targeted agent or chemotherapy is ongoing.

**Keywords:** LY3039478, Notch inhibition, gamma-secretase inhibitors metastatic cancer, colorectal cancer

Clinicaltrials.gov ID: NCT01695005
INTRODUCTION

The Notch signaling pathway plays an integral role in tissue development and homeostasis, and involve the inhibition of apoptosis and the promotion of cell proliferation [1] [2]. Deregulated Notch signaling due to mutation or overexpression of ligands and/or receptors is implicated in a number of malignancies [3-5]. LY3039478 is an orally bioavailable potent Notch inhibitor that prevents release of the Notch intracellular domain (NICTD) by inhibiting proteolytic activity of γ-secretase complex and thereby decreasing Notch signaling and its downstream biologic effects. LY3039478 has been shown to inhibit Notch signaling in cell lines representing a number of different solid tumors and leukemia including T-cell acute lymphoblastic leukemia. In xenograft models, LY3039478 demonstrates significant activity against human ovary, colon, and non-small-cell lung cancers [6].

After oral administration of 2.5- to 100-mg doses in preclinical study, LY3039478 is absorbed rapidly with time to maximum plasma drug concentration \((t_{\text{max}})\) occurring approximately 1 to 2 hours post dose. Exposures appear to increase in a dose-dependent manner. Renal clearance accounts for approximately 35% of total LY3039478 clearance, with elimination half-life \((t_{\frac{1}{2}})\) at approximately 4 to 6 hours.

Dosing regimen was established based on the schedule optimization studies in preclinical models (Supplementary Fig. 1). Nonclinical toxicology studies in rats and dogs dosed three times weekly (TIW) for 1 month have characterized the target tissues for toxicity that may be clinically relevant (data not shown). In nonclinical species, the gastrointestinal (GI) tract is the key target organ for toxicity, with some changes also observed in the reproductive systems. In the mouse GI system, administration of LY3039478 is associated with goblet cell hyperplasia and a mucoid enteropathy affecting the small and large intestine (Supplementary Fig. 2). This reversible enteropathy is characterized by development of nonformed or liquid mucoid feces.
In this phase I study, we have evaluated the safety, pharmacokinetic profile, pharmacodynamic effects, antitumor activity of LY3039478 in patients with advanced or metastatic cancer leading to the determination and confirmation of the recommended phase 2 dose.

METHODS

Study Design and Treatment

This phase I, open-label, multicenter, nonrandomized, and dose-escalation phase study enrolled 110 patients in 8 centers worldwide (ClinicalTrials.gov identified: NCT01695005). In part A, patients received increasing dose levels of oral LY3039478 (range: 2.5–100 mg) TIW on a 28-day cycle. Dose-escalation was guided by safety assessments from days 1 through 28 of cycle 1 for patients in all. Part B cohort expansion began following an interim review of the data at the MTD TIW on a 28-day cycle. LY3039478 was administered until symptomatic or confirmed progressive disease, unacceptable toxicity, or other reasons for study drug discontinuation.

The primary objectives of this study were to determine and confirm the recommended phase II dose that may be safely administered to patients with advanced or metastatic cancer and secondary objectives were to characterize the safety, tolerability, pharmacokinetic (PK) parameters, and preliminary antitumor activity of LY3039478.

The study was conducted in compliance with the Declaration of Helsinki, Council for International Organizations of Medical Sciences International Ethical Guidelines, International Council on Harmonisation Guidelines for Good Clinical Practice, and applicable local regulations. The protocol was approved by the ethics committees of all participating centers, and all patients provided written informed consent before study entry.

Patients
All patients had an Eastern Cooperative Oncology Group performance status score of 0 or 1, adequate organ and hematologic functions. Part B patients had measurable disease or reliable biomarker.

**Study Assessments**

**Safety Assessments**

All AEs and DLTs were coded according to the Medical Dictionary for Regulatory Activities, version 19.0 and were graded by National Cancer Institute’s (NCI) Common Terminology Criteria for Adverse Events (CTCAE) 4.0.

**Efficacy Assessments**

Radiographic imaging was conducted approximately every 8 weeks (2 treatment cycles). Depending on the histology, tumor responses were measured and recorded using the appropriate guidelines (RECIST 1.1 [7], and assessed using investigator determined response. Objective response rate (ORR) was the proportion of patients who achieved a CR or PR out of all the patients who received at least 1 dose of study drug. Best response was determined from a sequence of responses assessed. Change in tumor size was derived for all patients on therapy with measurable disease at baseline and at least 1 post-treatment assessment. Progression-free survival (PFS) time was measured from the start of treatment to the first date of progression of disease or of death from any cause.

Use of PET scan to assess treatment effect of LY3039478 was optional. Partial metabolic response by PET scan was defined as a minimum of 15% in tumor 18F-FDG SUV after one cycle of therapy, and greater than 25% after more than one treatment cycle, according to PET response criteria of the European Organization for Research and Treatment of Cancer [8].

**Pharmacokinetic Assessments**
LY3039478 plasma and urine concentrations were measured using validated LC/MS/MC methods.

**Exploratory Biomarker Assessments**

Gene expression: Skin biopsy samples collected before and after LY3039478 treatment were analyzed for changes in gene expression using a reverse transcriptase polymerase chain reaction (RT-PCR) method at Asuragen (Austin, TX, USA) per standard protocol [9]. Eighteen Notch signaling genes were analyzed: hairy and enhancer of split (Hes 1-7), Hes related with YPRW motif (Hey 1, 2 and L), olfactomedin 4 (OLFM 4), atonal bHLH transcription factor 1 (ATOH 1), deltex E3 ubiquitin ligase (DTX1/DTX2), Notch-regulated ankyrin repeat (NRARP), V-MYC Aavian myelocytomatosis viral oncogene and its neuroblastoma-derived homologs (MYC/MYCN), and Cyclin D1 (CCND1). TaqMan primer probes were procured from Applied Biosystems Life Technologies. Plasma Aβ levels were determined post-treatment on day 1 and day 22 of cycle 1.

Immunohistochemistry: A total of 68 specimens were submitted containing sufficient tumor tissue for biomarker evaluation following review by a board-certified pathologist (G.J.O) of a hematoxylin and eosin (H&E)-stained section of each sample (47 in part A and 21 in part B). Formalin-fixed paraffin-embedded (FFPE) specimens were sectioned at 4-5 μm and baked at 60°C for at least 15 minutes or until dry. Deparaffinization and antigen retrieval were accomplished at 97°C for 20 minutes with EnVision™ FLEX Target Retrieval Solution, high pH (K8000; Dako, Carpinteria, CA) on a Dako PT Link instrument. Separate sections of the patient’s tumor were assayed for the NICD specific for Notch 1, Notch 2 and Notch 3. Each specific intracellular domain was tested using neoepitope proprietary monoclonal antibodies specific for the Notch 1, Notch 2 and Notch 3 NICD, respectively, using Dako EnVision™ FLEX+ Rabbit Visualization System (K8009) performed on a Dako Autostainer Link 48 automated slide stainer.
Results were interpreted and scored qualitatively by a board-certified pathologist (G.J.O.) based on the immunoreactivity for the NICD fragment observed in the specimen using a scale of 0 (no staining) to 3+ (intense specific nuclear staining).

**Statistical Analyses**

Data from all patients who received at least 1 dose of LY3039478 treatment were included in summaries of safety and efficacy. Analyses of safety and efficacy are based on a July 2016 data transfer.

**Safety Analyses**

As study part A was a dose-finding study, data were analyzed on a cohort-by-cohort basis throughout the study until an MTD was determined. Dose escalation was driven by safety using the 3+3 method. Model-based analyses (a 2 parameter logistic model) [10] that incorporated prior expectations about the dose-toxicity curve were fitted to the data at the end of each cohort and were used to guide the next dose level. Safety analyses included summaries of drug exposure, DLTs and DLETs for all patients on therapy, preexisting conditions, TEAEs, discontinuations from the study because of AE or death, SAEs, CTCAE grades for laboratory and nonlaboratory AEs, and concomitant medications.

**Efficacy Analyses**

Efficacy analyses included descriptive summaries of ORR and PFS. The minimum change in tumor size and SUV\textsubscript{max} were summarized using a waterfall plot.

**Pharmacokinetic Analyses**

Pharmacokinetic parameter estimates for LY3039478 were calculated by standard noncompartmental methods of analysis and were evaluated to delineate effects of dose proportionality. Renal clearance was evaluated as an exploratory objective.
Pharmacodynamic Analyses

The Notch regulated gene expression was normalized using the geometric mean of housekeeping genes HPRT1 and PGK1. Percent inhibition was derived using the \(2^{-\Delta\Delta C_T}\) method [11]. Changes in plasma A\(\beta\) levels were evaluated by mixed effect Model Repeated Measures (MMRM) models. Data were log\(_e\)-transformed prior to analysis and the ratio to baseline evaluated with baseline included as a covariate, and fixed effect terms of treatment, day, time point and all interactions of treatment, day and time point. An AR(1) variance-covariance structure was used to account for repeated measures within a patient.

RESULTS

Patient Characteristics

In total, 110 patients with advanced or metastatic cancer were treated with LY3039478 monotherapy between 31 October 2012 and 15 July 2016. The median age of patients was 58 years and majority had ECOG PS 1 (71%). Patients were heavily pre-treated; 33% received \(\geq 5\) prior systemic therapy regimens. The most common tumor type are represented in Table 1. Of 47 tumors samples in part A and 21 in part B, 19 samples in part A and 11 samples in part B were positive for Notch 1, no samples were positive for Notch 2 or 3. The frequency of tumors staining positive for Notch 1 NICD per tumor type is presented in Supplementary Fig. 3.

Safety and Tolerability

During the dose-escalation portion of the study (part A), 5 of the 55 patients (9%) experienced dose-limiting toxicities (DLTs) (Supplementary Table 1). DLTs of grade 4 thrombocytopenia with bleeding were experienced by 1 patient each in cohort 4 (20 mg), cohort 5 (30 mg). Grade 4 thrombocytopenia without bleeding was observed in 1 patient in cohort 7 (60 mg). DLTs of grade 3 colitis (1 patient) and grade 3 nausea (not manageable with medical treatment) associated with grade 3 fatigue (1 patient) were experienced in cohort 9 (100 mg). In addition, 1
patient in cohort 7 (60 mg) experienced a dose-limiting equivalent toxicity (DLET) of grade 3 colitis during cycle 2, and 1 patient in cohort 9 (100 mg) experienced a DLET of grade 3 fatigue during cycle 4. The maximum tolerated dose (MTD) was determined to be 75 mg TIW. Diarrhea (n=25, 45%), and vomiting (n=25, 45%) were the most commonly reported treatment-emergent adverse events (TEAEs) possibly related to LY3039478 (Table 2). Median duration of treatment for patients in part A was 2 cycles (range: <1 to 18 cycles).

During the dose confirmation portion of the study (part B), additional review of the safety data from part B (25 patients) revealed that the 75-mg dose was not well tolerated, leading to dose reductions in approximately 30% of patients mainly due to GI toxicity.

In the dose confirmation phase, 25 patients received LY3039478 monotherapy at 75 mg, and 30 patients received LY3039478 monotherapy at 50 mg. The most commonly reported TEAEs possibly related to LY3039478 were diarrhea (n=33, 60%), vomiting (n=26, 47%), and nausea (n=24, 44%) (Table 3). With the reduction of the MTD of LY3039478 from 75 to 50 mg TIW, a lower incidence of diarrhea was observed (76% of patients vs. 47% of patients). At the MTD of 50 mg TIW the majority of the events were mild to moderate in severity, with few patients experiencing grade 3/4 events. Median duration of treatment for patients in part B was 2 cycles (range: <1 to 13 cycles). A total of 19 patients in part B experienced serious adverse events (SAEs) that were considered possibly related to the study drug by the investigator (Supplementary Table 2).

A total of 17 deaths have been reported on or within 30 days of study treatment. 15 patients died because of disease progression and 2 patients died due to an adverse event (AE) (1 patient received the 60 mg dose and 1 patient received the 100 mg dose, both due to sepsis).

**Pharmacokinetics**
Plasma exposure increased approximately dose proportionally (Supplementary Tables 3 and 4). Maximum concentrations were achieved approximately 2 hours post dose. The half-life is approximately 6 hours; no accumulation was noted with TIW dosing.

**Biomarker and Pharmacodynamic Analyses**

The effect of LY3039478 on plasma Aβ concentrations followed an approximate dose-dependent trend, with approximately 80% reductions in plasma Aβ observed from 45 to 100 mg (Supplementary Fig. 4).

Additionally, expression of Notch regulated genes was assessed to monitor pharmacodynamic effect of LY3039478 on skin. Skin samples were collected prior to and after approximately 6 hours of LY3039478 treatment. The panel consisted of Notch-regulated genes, of which inhibition ≥50% was observed for hairy and enhancer of split-1 (Hes1), cyclin D1 (CCND1), and Notch-regulated ankyrin repeat (NRARP)], (Fig. 1). The effect of LY3039478 on levels of other genes was weak to none (Hes2, Hes4, Hes5, HeyL and MYC). The maximum PD effect was observed at 45 mg and was maintained throughout the dose escalation, suggesting the 45 mg dose may be the minimal biological efficacious dose.

**Antitumor Activity**

In part A, partial response was observed in 1 patient with breast cancer (ER/PR+ HER2- and FBXW7 mutation), with duration of response of 9.5 months. Seventeen (31%) patients had a Best response of stable disease is observed in 17 (31%) and 19 (35%) patients of part A & B, respectively, of which sustained duration >6 months was observed in 5 patients in each part. In Part B, no patients had complete response (CR) or partial response (PR). The change in tumor size for patients who had measurable disease and at least one post treatment radiological assessment (39 patients in part A and 41 patients in part B) is shown in Fig. 2. This summary is consistent with the summary of ORR as reported by the investigators (1 patient in part B had a
100% decrease in tumor size for their target lesion, but progressive disease due to appearance of new lesions. In addition, we did observe tumor necrosis in some patients. Scans from patients with various tumor types are shown in Supplementary Fig. 5.

Across both dose escalation and dose expansion study parts for which patients with advanced cancers of multiple histologies were enrolled, the estimated median time to disease progression or death was 1.68 months (95% CI: 1.45–2.04) and 1.77 months (CI: 1.68–3.29), respectively. Thus far, emerging unvalidated results for \( ^{18}\text{F-FDG} \) positron emission tomography/computed tomography (PET-CT) assessment included 2 metabolic responders in patients with adenoid cystic carcinoma and testis cancer (metastatic malignant Sertoli-Leydig tumor). In part A, 1 (chondrosarcoma) of 10 patients with pre- and post-treatment PET-CT assessment had unconfirmed partial metabolic response, and 22 of 55 patients in part B with pre- and post-treatment PET-CT assessment, of which 4 patients (breast cancer, glioblastoma, rectal cancer, and adenoid cystic carcinoma) had unconfirmed partial metabolic response (Fig. 3).

Pre and post treatment biopsies were obtained from a patient with leiomyosarcoma receiving 100 mg LY3039478 (Fig. 4). Histologically, there is extensive necrosis of the tumor on the post-treatment biopsy. These changes were seen in association with a rapid radiographic response to LY3039478 monotherapy identified on a CT scan obtained on cycle 1 day 4 of treatment. Immunohistochemistry performed on pre- and post-treatment biopsies shows this leiomyosarcoma was positive for activated Notch 1 receptor.

**DISCUSSION**

This report describes the first-in-human treatment with LY3039478, a highly potent and selective Notch inhibitor. We have established LY3039478 can be dosed safely on a TIW schedule. Five DLTs were identified, and the recommended phase dose was determined to be 75 mg TIW and then reduced to a dose of 50 mg TIW. Gastrointestinal toxicity (diarrhea and nausea) was the
major toxicity observed in this study. These events were consistent with the previously reported clinical safety profile for Notch pathway inhibitors and are mechanism based [12-19]. With the reduction of the MTD of LY3039478 from 75 to 50 mg TIW in the dose confirmation phase, a lower incidence of diarrhea was observed, suggesting a positive effect on GI tolerance.

The PK parameters of LY3039478 increased in a dose-proportional manner. Plasma Aβ (a surrogate PD marker for Notch signaling following administration of a γ-secretase inhibitor) [20] and Notch pathway gene expression measurements showed that 80% of maximal biomarker effects were obtained at approximately 15 to 50 mg LY3039478. This substantial Notch pathway inhibition supports the use of the 50 mg TIW LY3039478.

Although the patient population was heterogeneous and heavily pretreated (33% of patients receiving LY3039478 monotherapy as sixth line or greater), 1 PR and 10 instances of prolonged stable disease were achieved. In addition, metabolic responses or tumor necrosis was observed in other patients. Of note, 3 patients with adenoid cystic carcinoma had a duration of treatment of 8.0 months, of which one was evaluable for IHC and was Notch-1+. This result correlates with recent prevalence studies revealing adenoid cystic carcinomas have a high expression of Notch-1 receptor activation [21].

The results of this trial demonstrate the safety and preliminary antitumor activity of LY3039478 as a single agent. A major strength of the presented results is the clinical pharmacodynamic effect seen on Notch targeted genes after treatment with LY3039478. This work supports the rational for targeting Notch signalling and further implicates aberrant Notch signalling in tumor physiology. Further investigation with LY3039478 as monotherapy and in combination with targeted agent or chemotherapy is ongoing in a variety of tumor types.
Disclosure of Potential Conflicts of Interest


Authors’ Contributions

All the authors have contributed substantially to the data analysis and interpretation, and the drafting of the manuscript. Christophe Massard, Claire Smith, Ute Ohnmacht, Karim A. Benhadji and Jordi Rodon contributed to study design. Claire Smith, Ute Ohnmacht, Gerard Oakley, Bharvin K.R. Patel, Eunice S.M. Yuen, Karim A. Benhadji contributed to data analysis. Christophe Massard, Analía Azaro, Jean-Charles Soria, Ulrik Lassen, Christophe Le Tourneau, Debasish Sarker and Jordi Rodon contributed to data collection.

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HES1 (A), CCND1 (B), NRARP (C), HES2 (D), HES4 (E), HES5 (F), MYC (G), HEYL (H) Genes were normalized by HPRT1 and PGK housekeeping genes. The % inhibition for post-treatment values relative to pre-treatment values is illustrated using boxplots.

**Figure 2.** Change in tumor size for patients who had measurable disease and at least one post-treatment radiological assessment.

**Figure 3.** Change in SUV$_{\text{max}}$ by PET assessment.

**Figure 4.** Biopsy from patient with leiomyosarcoma treated with LY3039478 (100 mg), staining for NICD.
<table>
<thead>
<tr>
<th>Table 1. Patient and Disease Characteristics</th>
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<tr>
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Table 2. Treatment-emergent adverse events (possibly drug-related) occurring in ≥10% patients in the dose escalation phase

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<td>0 0 1 0</td>
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<td>0 0 0 0</td>
<td>0 0 0 0</td>
<td>0 0 0 0</td>
<td>2 1 0 0</td>
<td>2 0 0 0</td>
<td>6 (11%)</td>
</tr>
<tr>
<td>Metabolism and nutrition disorders</td>
<td>Dry Skin</td>
<td>1 0 0 0</td>
<td>0 1 0 0</td>
<td>0 0 0 0</td>
<td>1 0 0 0</td>
<td>0 0 0 0</td>
<td>1 0 0 0</td>
<td>1 0 0 0</td>
<td>2 0 0 0</td>
<td>0 1 0 0</td>
<td>8 (15%)</td>
</tr>
<tr>
<td></td>
<td>Hair Color changes</td>
<td>1 0 0 0</td>
<td>1 0 0 0</td>
<td>1 0 0 0</td>
<td>2 0 0 0</td>
<td>1 0 0 0</td>
<td>1 0 0 0</td>
<td>0 0 0 0</td>
<td>1 0 0 0</td>
<td>1 0 0 0</td>
<td>9 (16%)</td>
</tr>
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</table>
Table 3. Treatment-emergent adverse events (possibly drug-related) occurring in ≥10% patients in the dose confirmation phase

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<th>Type of toxicity</th>
<th>Toxicity</th>
<th>50 mg (N=30)</th>
<th></th>
<th></th>
<th></th>
<th>75 mg (N=25)</th>
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<th></th>
<th></th>
<th>Total N=55</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>Grade</td>
<td>1 n (%)</td>
<td>2 n (%)</td>
<td>3 n (%)</td>
<td>4 n (%)</td>
<td>1 n (%)</td>
<td>2 n (%)</td>
<td>3 n (%)</td>
<td>4 n (%)</td>
<td>n (%)</td>
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<tr>
<td>Gastrointestinal disorders</td>
<td>Diarrhea</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td>8 n (%)</td>
<td></td>
<td></td>
<td>8 n (%)</td>
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</tr>
<tr>
<td></td>
<td>Dry Mouth</td>
<td></td>
<td>3 (10)</td>
<td></td>
<td>1 (3)</td>
<td></td>
<td></td>
<td>2 (8)</td>
<td></td>
<td>1 (4)</td>
<td></td>
</tr>
<tr>
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<td></td>
<td></td>
<td></td>
<td>7 (13)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Nausea</td>
<td></td>
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<td></td>
<td>4 (13)</td>
<td></td>
<td></td>
<td>5 (20)</td>
<td></td>
<td>7 (28)</td>
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<td></td>
<td></td>
<td>1 (4)</td>
<td></td>
<td></td>
<td>6 (11)</td>
</tr>
<tr>
<td></td>
<td>Vomiting</td>
<td></td>
<td>10 (33)</td>
<td></td>
<td>4 (13)</td>
<td></td>
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<td>1 (4)</td>
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<tr>
<td>Nervous system disorders</td>
<td>Dysgeusia</td>
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<td></td>
<td>1 (4)</td>
<td></td>
<td>1 (4)</td>
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<tr>
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<td>3 (10)</td>
<td></td>
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<td>4 (16)</td>
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<tr>
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<td></td>
<td>1 (4)</td>
<td>1 (4)</td>
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<tr>
<td></td>
<td>Pyrexia</td>
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<td></td>
<td>2 (8)</td>
<td></td>
</tr>
<tr>
<td>Metabolism and nutrition disorders</td>
<td>Decreased Appetite</td>
<td></td>
<td>4 (13)</td>
<td></td>
<td>6 (20)</td>
<td></td>
<td></td>
<td>3 (12)</td>
<td></td>
<td>3 (12)</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hypophosphatemia</td>
<td></td>
<td>2 (7)</td>
<td></td>
<td>2 (7)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2 (8)</td>
<td>3 (12)</td>
</tr>
<tr>
<td>Skin and subcutaneous tissue disorders</td>
<td>Alopecia</td>
<td></td>
<td>3 (10)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>3 (12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Dry Skin</td>
<td></td>
<td>3 (10)</td>
<td></td>
<td>2 (7)</td>
<td></td>
<td></td>
<td>3 (12)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Hair Color changes</td>
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<td>4 (13)</td>
<td></td>
<td></td>
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<td></td>
<td>2 (8)</td>
<td></td>
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</table>
Figure 1. Gene Expression Summary.

HES1 (A), CCND1 (B), NRARP (C), HES2 (D), HES4 (E), HES5 (F), MYC (G), HEYL (H) Genes were normalized by HPRT1 and PGK housekeeping genes. The % inhibition for post-treatment values relative to pre-treatment values is illustrated using boxplots.
Figure 2. Change in tumor size for patients who had measurable disease and at least one post-treatment radiological assessment.

**Part A**

**Dose (mg)**

- 2.5
- 5
- 10
- 20
- 30
- 45
- 60
- 75
- 100

**Change from baseline (%)**

Patients (n = 39)

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**Part B**

**Dose (mg)**

- 50
- 75

**Change from baseline (%)**

Patients (n = 41)
Figure 3. Change in $\text{SUV}_{\text{max}}$ by PET assessment

**Part A**

Change from baseline (%)

Patients ($n = 10$)

**Part B**

Change from baseline (%)

Patients ($n = 22$)
Biopsy from patient enrolled with leiomyosarcoma in the 100 mg cohort of dose escalation. A) shows the patient’s tumor prior to LY3039478 monotherapy (H&E, 100x total magnification) and B) shows the same tumor after radiographic images demonstrating greater than 90% necrosis of the tumor on cycle 1 day 2 (H&E, 100x total magnification). Histologically, there is extensive necrosis and occasional apoptotic debris with few surviving tumor cells. C) shows the patient’s tumor prior to LY3039478 was positive for Notch 1 activation (NICD Notch 1 IHC, 100x total magnification), while D) shows the few remaining tumor cells are positive for Notch 1 activation (NICD Notch 1 IHC, 100x total magnification). The intensity of the surviving tumor cells in panel D) is at least as strong for Notch 1 as the high intensity pre-dose tumor cells in panel C).