Tumor Survivin is Downregulated by the Antisense Oligonucleotide LY2181308: 
A Proof of Concept, First-in-Human Dose Study

Denis C. Talbot¹, Malcolm Ranson², Joanna Davies¹, Michael Lahn³, Sophie Callies⁴, Valérie André⁴, Sunil Kadam³, Michael Burgess⁴, Christopher Slapak³, Anna L.Olsen¹, Peter J. Mchugh¹, Johann S. de Bono⁵, Julian Matthews⁶, Azeem Saleem⁶, Patricia Price⁷

¹ University of Oxford Department of Medical Oncology, Oxford Radcliffe Hospitals NHS Trust, UK
² School of Cancer and Enabling Sciences, MAHSC, University of Manchester, Christie Hospital NHS Foundation Trust, Manchester, UK
³ Early Oncology Clinical Investigation, Eli Lilly & Co, Indianapolis, IN, USA
⁴ Eli Lilly & Co, Erl Wood Research Centre, Windlesham, UK
⁵ Institute of Drug Development, Royal Marsden, Sutton, UK
⁶ Wolfson Molecular Imaging Centre, MAHSC, University of Manchester, UK
⁷ Academic Radiation Oncology, The Christie Hospital, National Health Services (NHS) Foundation Trust, University of Manchester, UK
Statement of Translational Relevance

Evasion of apoptosis is a hallmark of malignancy. Survivin is a small naturally occurring inhibitor of apoptosis (IAP) that is often highly expressed by cancer cells making it an attractive target for therapeutic intervention. The antisense oligonucleotide (ASO) LY2181308 down-regulates survivin by targeting the survivin transcript and has anti-tumor efficacy through induction of tumor cell apoptosis in pre-clinical models. This First-In-Human (FHD) study demonstrates the proof of concept that antisense oligonucleotide therapy directed against survivin mRNA reduces survivin mRNA and protein levels and restores apoptosis in tumors of cancer patients. The research represents a critical step in the translation from discovery of survivin, an important tumor related IAP, to successful clinical application of survivin-targeted therapy. Finally, this FHD study helps to better understand how ASOs can be used as novel agents in the treatment of cancer.
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Conflict of Interest Statement:

The authors Michael Lahn, Sophie Callies, Valérie André, Sunil Kadam, Michael Burgess, Christopher Slapak are employees of Eli Lilly and Company, are fully compensated and hold stock in the company.

Except for Denis C. Talbot (see attached form for Conflict of Interest), all other academic authors have no conflict of interest to declare: Malcolm Ranson, Joanna Davies, Anna L. Olsen, Peter J. McHugh, Johan De Bono, Julian Matthews, Azeem Saleem, Patricia Price

Corresponding Author:

Dr. Denis C. Talbot

University of Oxford Medical Oncology Department

Cancer and Haematology Center

Churchill Hospital, Old Road, Headington

Oxford, OX3 7LJ

United Kingdom

Tel: +44 (0)1865 235311

Fax: +44 (0)1865 235985

Email: denis.talbot@medonc.ox.ac.uk
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Abstract

Purpose:
Enhanced tumor cell survival through expression of inhibitors of apoptosis (IAP) is a hallmark of cancer. Survivin, an IAP absent from most normal tissues, is over-expressed in many malignancies and associated with a poorer prognosis. We report the First-in-Human dose study of LY2181308, a second generation antisense oligonucleotide (ASO) directed against survivin mRNA.

Patients and Methods
A dose-escalation study evaluating the safety, pharmacokinetics and pharmacodynamics of LY2181308 administered intravenously for 3-hours as a loading dose on three consecutive days and followed by weekly maintenance doses. Patients were eligible after signing informed consent, had exhausted approved anti-cancer therapies and agreed to undergo pre- and post-treatment tumor biopsies to evaluate reduction of survivin protein and gene expression.

Results
A total of 40 patients were treated with LY2181308 at doses of 100 to 1000 mg. Twenty-six patients were evaluated at the recommended Phase 2 dose of 750 mg, at which level serial tumor sampling and $^{11}$C-LY2183108 PET imaging demonstrated that ASO accumulated within tumor tissue, reduced survivin gene and protein expression by 20% and restored apoptotic signaling in tumor cells in vivo. Pharmacokinetics were consistent with pre-clinical modeling, exhibiting rapid tissue distribution and terminal half-life of 31 days.

Conclusion
The tumor-specific, molecularly targeted effects demonstrated by this ASO in man underpin confirmatory studies evaluating its therapeutic efficacy in cancer.
Introduction
Survivin, a 16.5 KDa protein encoded by the essential gene BIRC5, was originally identified as an inhibitor of apoptosis (IAP) that exerts its effects through binding to SMAC (second mitochondrial activator of caspases) preventing the sequestration of IAPs by SMAC and inhibition of caspase dependent apoptosis (1). As a component of the kinetochore-associated complex, survivin also plays an important role in the regulation of late mitosis and cytokinesis (2). Survivin is expressed in a wide range of human cancers, and when over-expressed is associated with a poorer prognosis (3). With the exception of placenta, thymus, activated T cells, gastrointestinal crypt cells and regenerating liver, survivin is not expressed in normal adult tissue (4). Thus survivin represents an attractive molecular target for therapeutic intervention. Targeted approaches against survivin include small molecule inhibitors against the survivin protein, gene silencing and survivin mRNA blockade (5, 6). The 2’-O-methoxyethyl modified antisense oligonucleotide (2nd generation ASO) LY2181308 is a 18-mer ASO, that binds to the translation initiation codon of the survivin transcript. Following ASO hybridization to survivin mRNA, RNaseH-dependent cleavage of the duplex ensues with subsequent degradation of the survivin mRNA (7). Thus, survivin protein expression is specifically inhibited without affecting expression of other genes including other IAP proteins (8). Compared to phosphorothioate or 1st generation ASOs, 2nd generation ASOs are more stable, have an improved pharmacokinetic profile, increased potency and reduced toxicity (9). Using aggregate data from pre-clinical pharmacology and toxicology, an integrated PK/PD model was developed to predict a biologically effective dose range for clinical evaluation (10). Based on this, a First-in-Human Dose (FHD) study was designed to evaluate the bio-distribution profile of LY2181308 by measuring the following tumor specific pharmacodynamic changes: tumor tissue penetration of
LY2181308, down-regulation of tumor survivin at the mRNA and protein levels and restoration of tumor apoptosis at a dose and schedule that was safe in humans. To this end, pre- and post-treatment tumor sampling was performed for rigorous assessment of target modulation (i.e., survivin protein and gene expression), the effect of restoring normal apoptosis/cell cycle-related protein activity and biodistribution of LY2181308, including sub-cellular localization.
Patients and Methods

Study Design: This FHD monotherapy study was divided into three parts: Part A (safety, PK), one-patient-cohorts with initial 100 mg and escalated, by dose doubling, to 400 mg; Part B (safety, PK, PD), three-patient-cohorts with 50% dose escalation until dose limiting toxicity (DLT); Part C (dose confirmation cohort, PK, PD). The transition from Part A to Part B was planned on the basis of either development of toxicity or evidence from PK that the predicted biological effective dose (BED) had been reached. The recommended dose for phase II studies was determined from both DLT and the reduction in survivin expression in tumor tissue at the anticipated BED as defined by the preclinical PK/PD model of Callies et al. (10). At the recommended Phase 2 dose, two companion studies were conducted at the Universities of Oxford (Study 1, endobronchial tumor sampling) and Manchester (Study 2, [11C] LY2181308 study). The entire study was approved by the Medicines and Healthcare products Regulatory Agency (MHRA) and a Multicentre Research Ethics Committee (MREC).

Enrollment Criteria: Inclusion criteria: at least 18 years of age, confirmed malignancy, exhausted approved standard therapies, tumor accessible for biopsy, written informed consent, Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1, discontinued previous anti-cancer therapies, absolute neutrophil count (ANC) ≥1.5 x 10^9/L, platelets ≥100 x 10^9/L, haemoglobin ≥9g/dL, normal serum bilirubin, less than 2.5x upper limit of normal alanine transaminase (ALT) and aspartate transaminase (AST), calculated creatinine clearance ≥50 mL/min, normal activated prothrombin time (aPTT) and prothrombin time (PT), and contraceptive precautions taken. Exclusion criteria: bleeding diathesis, major surgery within last 4 weeks, pregnant or lactating, symptomatic central nervous system (CNS) neoplasm, taking...
concomitant anticoagulant therapy, received prior ASO, treatment with an unapproved drug, positive test results for viral infection.

**Drug formulation and schedule of administration**: LY2181308 was diluted in 500 mL of normal saline for intravenous injection and infused as a 3-hrs infusion on three three consecutive days as a loading dose, followed by weekly maintenance doses. The dose and dose schedule were based on pre-clinical safety, PK and pharmacology studies of LY2181308. These were integrated within a PK/PD model that predicted the BED (10).

**Treatment Assessment**: DLT was defined (CTCAE version 3.0) (11) as one out of three patients with the following: Grade 3/4 hematological toxicities >5 days, aPTT prolongation >48 hrs after infusion, any Grade 3/4 non-hematological toxicity or any other DLT, including those associated with sustained complement activation. Laboratory examinations: CRP, complement split products (National Jewish Medical and Research Center, Denver, CO), hematology, serum chemistry. Pre-treatment tumor biopsy: after enrollment and before first loading. Post-treatment tumor biopsy: window of 48 to 96 hours after the third loading dose. Descriptive statistics were used for all patients receiving at least one dose of LY2181308 to evaluate safety, PK and efficacy.

**Pharmacokinetic (PK) Assessment**: PK parameters were analysed (WinNonlin Enterprise, Version 5.2) for: time of maximum concentration (Tmax), maximum plasma concentration (Cmax), area under the plasma concentration versus time curve (AUC), and clearance (CL) following the third and fifth administration of LY2181308 on Day 3 and Day 15. Plasma PK data were pooled for non linear mixed affect modelling analysis (NONMEM, Version 6.1) to determine the compartmental PK parameters: mean value, variance, subject variability.
inter-individual variability was coded as an exponential model and the residual variability as a proportional model.

**Development of a Specific Antibody to Detect the ASO:** A polyclonal rabbit antibody against the ASO was generated using a KLH modified ASO (Lampire Biological Laboratories, Pipersville, PA, USA). It was validated for use in formalin-fixed, paraffin-embedded specimen utilizing Ventana’s DiscoveryXT™ staining platforms (Ventana, Tucson, AZ, USA).

**Pharmacodynamic Assessment:** Formalin-fixed tissue was used for IHC assessment at Ventana (Tucson) to determine: ASO, survivin (NB500-201, Novus Biologicals, Littelton, CO, USA), cleaved caspase 3 (CST9661, Cell Signaling Technologies, Danvers, MA, USA), and Ki67 (mouse monoclonal antibody, Ventana). All antibodies were detected by appropriate secondary antibodies (Vector Laboratories, Burlingame, CA, USA). Staining intensity was determined using the HSCORE and Ventana’s automated imaging software (12). Gene expression analysis (Panomics, Fremont, CA, USA) (13) was used to quantify Survivin mRNA expression (branched DNA, bDNA). Percentage change in protein and mRNA expression was summarised using medians and approximate 95% confidence limits (14).

**Endobronchial Biopsy:** Fiberoptic endobronchial tumor sampling is a safe, short, standard procedure (15). Endobronchial tumors were visualized and sampled by brushing with a 1 mm brush. The samples were processed immediately for flow cytometry assessment.

**Flow Cytometry:** Briefly, disaggregated tumor cells were fixed in formadehyde and subsequently stained with Phycoerythrin-conjugated monoclonal antibodies directed against survivin (Clone 91630, R&D Systems, Abingdon, UK). Flow cytometric data (obtained on a CyAN machine, Dako, Denmark) was analyzed using Summit software (Dako).
[\textsuperscript{11}C]LY2181308 PET imaging: After establishing safety in non-human primates (16), patients received <1mg and <600MBq of [\textsuperscript{11}C]LY2181308 prior to LY2181308, and during the maintenance infusion on Day 15 (17). PET data was collected for 90 minutes following bolus radiotracer injection.

FDG-PET Imaging: FDG-PET imaging was performed prior to treatment with LY2181308 and on day 22. The imaging was performed as previously recommended (18) and consistent with institutional radiation guidelines.
Results

Between October 2004 and December 2008, 40 patients were enrolled in this monotherapy FHD study, of whom 17 were enrolled in the initial dose escalation stage with doses ranging from 100 mg to 1000 mg. Twenty-six patients were treated at the 750 mg dose level, including 6 patients in two site-specific studies: Study 1 assessed apoptosis and cell cycle progression changes by flow cytometry of endobronchial tumor cells in 3 patients with NSCLC; Study 2 examined the biodistribution of [11C]LY2181308 measured by PET in 3 patients. The demographics of the study population was typical of Phase 1 oncology trials (19) with the majority of patients having received prior chemotherapy (Table 1). Fourteen patients with hepatic metastasis were included, of whom 11 had their biopsy taken from their liver lesions. LY2181308 was well tolerated with the majority of the patients showing Grade 1 or 2 toxicities (29/40, 72.5%). Out of the 40 patients, 11 patients (27.5%) had Grade 3 or 4 toxicities (Table 2). Two of the 11 patients received the 1000 mg dose. One patient had a Grade 3 hypophosphatemia, while the other patient experienced a Grade 4 lymphopenia and a prolonged Grade 3 headache, which did not respond to pain medication. This event was defined as the dose limiting toxicity (DLT) for this study. Concurrently, a sharp rise in C-reactive protein (CRP) was observed in this patient raising the concern of a possible complement-induced cerebrospinal leak-syndrome. Three patients were treated at the 900 mg dose level and no DLTs were observed, but CRP, AST/ALT and moderate flu-like symptoms occurred during the loading doses, suggesting continued complement-activation. The most common symptoms were flu-like syndrome (fever, rigor, muscleskeletal pain, nausea), fatigue and vomiting (Table 2). The most frequent laboratory toxicity was prolongation of aPTT (generally Grade 1), which was observed at the time of the infusion in 75% of the patients. Other laboratory abnormalities, not considered as severe adverse events by
investigators, included lymphopenia (70%), thrombocytopenia (38%), hypokalemia (38%) and anemia (35%). Transient increases in complement Bb were noted at the end of the third loading dose (Supplemental Figure 1) but was not associated with adverse clinical events. Given the comparable PK profile of the higher doses of 900 and 1000 mg to the 750 mg dose (Supplemental Fig 2), the recommended dose of LY2181308 for further clinical studies using the same dosing schedule was defined as 750 mg, a dose likely to be safe in combination with chemotherapeutics.

The PK properties of LY2181308 and reduction of survivin expression in tumor tissue were assessed by collecting plasma samples and tumor biopsies before and after completing the loading dose. In parts B and C of the protocol, 31 patients were enrolled with 26 (84%) agreeing to have both pre- and post-treatment biopsies. Ten patients had either insufficient tumor material in the post-treatment biopsy or endogenous pigments (bilirubin, hemosiderin and melanin) that interfered with IHC staining. Hence, in 16 (52%) patients, we obtained sufficient pre- and post-dosing tumor tissue to assess the pharmacodynamic effects of LY2181308 by protocol-defined laboratory procedures (Figure 1, a-f).

Survivin protein expression as measured by IHC was reduced by 21%, concomittant with a statistically significant 20% reduction in mRNA expression (p<0.05) as shown by gene expression analysis (Fig 1g). Because of the high intensity of nuclear survivin IHC staining prior to the treatment, reduction of nuclear protein expression was more clearly detected than changes seen in the cytoplasm (Figure 1, e-g). Similarly, in the site-specific study in NSCLC, a reduction of survivin protein expression was demonstrated by flow cytometry in endobronchial tumor cells compared to pre-treatment levels in all 3 patients studied (Figure 1, i).
The PK of LY2181308 confirmed a multiphasic disposition in plasma with rapid tissue distribution half-lives of 30 min, 2.5 hrs, 12 hours and an elimination half-life of 31 days (Figure 2a). The terminal half-life was the result of a clearance of 20-30 L/h and volume of distribution of >1000 L consistent with the tissue distribution of LY2181308 of approximately 90%. As described for other ASOs (9), the primary sites of normal tissue uptake of LY2181308 were renal and hepatic, with less uptake seen in other normal tissue (17). The ASO was detected by IHC in tumor tissue in 10 of 11 patients, either within tumor cells or tumor-associated macrophages (Fig 2, b, c), and in 5 of 11 patients in stroma (Fig 2, d,e). Compatible with the IHC staining, the $^{[1]}$CLY2181308 PET imaging confirmed that following administration of 1 mg of $^{[1]}$CLY2181308, tumor penetration of LY2181308 ranged from 21 to 84 ng*h/mL tumor with a maximum concentration that ranged from 10 to 60 ng/mL. These levels are comparable with the concentration measured by ELISA in tumor biopsy and with that required for target inhibition (Table 3). The PK profile and tissue distribution of LY2181308 were consistent with the PD effects of the agent described above. However, there was no evident relationship between the localization of the ASO within the tumor tissue compartments, clinical response or change in survivin expression, perhaps due to the limited numbers of patients included within each cohort.

Of 22 patients assessed for efficacy using the RECIST criteria (20), a total of 4 patients achieved stable disease, including one patient with metastatic melanoma who remained free of disease progression for 18 months. Reduction of survivin expression was associated with an increase in the apoptosis marker cleaved caspase 3 (CC3) and a reduction in tumor expression of the proliferation marker Ki67 and (Figure 3a, b). In the single patient assessed, a partial metabolic response in several mesothelioma lesions was observed with a 40% reduction in standard uptake values (SUV) on FDG-PET imaging (21) (figure 3c, d).
Discussion

Few FHD studies of ASOs have sought to demonstrate pharmacodynamic changes in cancer patients at safe doses, such as reduction of the targeted mRNA and protein levels in tumor tissue (22-25). This FHD study of the 2nd generation ASO LY2181308 was designed to include analysis of serial tumor samples to prove the concept that the ASO was able to inhibit the function of survivin at a safe dose and schedule. We show that the ASO accumulated in tumor tissue, significantly downregulated tumor survivin mRNA and protein expression and enhanced the expression of markers indicative of restored tumor cell apoptosis. Consistent with previous studies, serial tumor biopsy procedures were safe and accepted by patients (26). Analysis of pre- and post-treatment biopsies demonstrated a reduction of tumor mRNA and protein expression by approximately 20% in a wide range of tumor types. This reduction is lower than that seen in xenograft models, where up to 50% reduction in survivin mRNA and protein were reported (10). In five cases, the presence of melanin, bilirubin or hemosiderin in tumor tissue interfered with the IHC staining and consequently changes in survivin levels after treatment with LY2181308 could not be determined. Tumor samples obtained from patients with NSCLC using fiberoptic bronchoscopy were perhaps even more effective in demonstrating survivin reduction. However, a larger number of patients would need to be included in future studies to confirm our observation. Although survivin levels were reduced, objective clinical responses were not observed and thus direct cytotoxic effects on tumors were difficult to determine. This was an expected finding in the context of a FHD study and because pre-clinical data suggested that LY2181308 has a cytostatic, rather than a cytotoxic, anti-tumor effect. Supporting this mode of action were the observations that one patient with metastatic melanoma had stable disease whilst receiving maintenance LY2181308 weekly for 18
months, and one patient who had a partial metabolic tumor response based on FDG PET imaging. Whilst anti-tumor efficacy was not a primary end point of the study, it was disappointing that no objective responses were seen. Whether this would have been achieved had survivin mRNA and/or protein levels been reduced by more than 20% can not be addressed by the current study. Combination studies of LY2181308 will assess whether inhibition of survivin enhances anti-tumor effects of pro-apoptotic agents such as docetaxel.

An important goal of this study was to demonstrate that pharmacodynamic responses were consistent with the predicted pharmacokinetic profile of LY2181308 in plasma and tissue (Table 3) and its similarity with other 2\textsuperscript{nd} generation ASOs (9). This prediction was confirmed at the 750 mg dose (Table 3). This is consistent with the observation that ASOs can be scaled successfully from animal to human. The PK profile of LY2181308 and its terminal half-life of about 30 days require a weekly maintenance dose to keep tissue concentrations at levels that were associated with target inhibition in animals. We also demonstrate that the ASO penetrates tumor tissue as detected by IHC and quantitative assessment of ASO levels by ELISA (Table 3). The simulated concentrations in the tumor tissue were confirmed by concentration measurements using ELISA and the \([^{11}\text{C}]\)LY2181308 study (Table 3). The range of LY2181308 concentration level observed in the tumor is slightly lower though overlapping relative to the range of LY2181308 trough plasma concentration. This could be explained by the fact that other tissues such as liver and kidney, in addition to the tumor, contribute to the equilibrium between LY2181308 tissues and plasma concentrations. The advantage of employing IHC is that it was possible to localize both the disposition of the ASO within tumor tissue and its intra-cellular distribution. There appeared to be a difference in localization of the ASO within tumor cells and the tumor microenvironment. Whether this observation was a result of the wide range of tumor...
types studied, the site of the biopsy or the timing after the last dose of LY2181308 is unclear (15 patients had their biopsy taken on Day 4; 8 on Day 5; 1 on Day 6 and 2 on Day 7). The variance in timing of post-treatment biopsies resulted in a degree of heterogeneity of results and may have introduced bias. Future studies should define consistent timing of biopsies for all patients and study sites. The use of \[^{11}C\]LY2181308 PET further supported the IHC-based observation that the ASO penetrated tumor tissue at a pharmacologically relevant concentration. Hence, LY2181308 clearly accumulated in tumor tissue over time (17). This is consistent with studies in animals, where the distribution and stability of the 2\(^{nd}\) generation ASOs was evaluated (9, 27). Other FHD studies evaluating inhibitors of the IAP family have not reported similar pharmacodynamic changes in solid tumor tissue after dosing with ASOs. For example, the small molecular weight survivin inhibitor YM155 was only evaluated for safety and pharmacokinetics (28), while in the clinical trial of the XIAP ASO inhibitor AEG35156 pharmacodynamic changes in PBMCs were reported in addition to safety and pharmacokinetics (25). Furthermore, the 1\(^{st}\) generation ASO oblimersen was evaluated for pharmacodynamic activity in melanoma patients only, but the proposed dose and dose schedule was not chosen for future Phase 2 studies (22). The 2\(^{nd}\) generation ASO OGX-011 against clusterin did evaluate pharmacodynamic changes in prostate cancer tumor tissue and is the only trial that established a dose and dose regimen based on pharmacodynamic activity in cancer patients that was later used in phase 2 studies (23).

Finally, the dose range at which we observed consistent survivin reduction in tumor tissue had a favorable toxicity profile for future clinical development. Grade 3 and 4 toxicities were present in 11 patients (27.5%) and acute renal failure was not observed during the first cycles of treatment as reported for YM-155, a small molecule survivin inhibitor (28). However, we did observe increased creatinine values (Grade 2), which returned to baseline levels after stopping...
the agent in one patient with metastatic melanoma who received LY2157299 at the 750 mg dose for 18 months (29). Although several confounding factors were present, we cannot exclude that LY2157299 treatment was associated with this reversible renal injury. While not considered medically adverse, lymphopenia was seen in 70% of the patients (Supplemental Figure 1c). Whether this was a pharmacodynamic effect of LY2181308-induced apoptosis of survivin-expressing lymphocytes (30) or reflected an off-target effect of the ASO will require additional investigation. A similar effect on lymphocyte counts was seen with the ASO AEG35156 against XIAP (25). Typical off-target effects of ASO administration were observed for LY2181308 including anemia, thrombocytopenia and transient prolongation in aPTT (31). In contrast to the phosphothioate ASOs or high doses of 2nd generation ASOs, the off-target toxicity of LY2181308 were milder and generally limited to Grade 1 and 2. This favorable toxicity profile was also recently confirmed in Japanese patients, in particular the loading dose-associated elevation of complement Bb (32).

In conclusion, the integration of pharmacodynamic and pharmacokinetic analyses in this FHD study has provided the proof of concept of effective and specific down-regulation of the key molecular target, survivin, in tumor tissue by the second generation ASO, LY2181308. These findings validate the application of 2nd-generation ASOs for cancer patients. The 750 mg dose and schedule of LY2181308 is currently being evaluated in clinical studies in combination with agents that induce apoptosis, such as chemotherapy or radiation (33, 34).
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Figure Legend

Figure 1. Reduction of survivin protein and mRNA expression after loading dose of LY2181308. IHC of tumor biopsy from one representative patient with breast cancer (a-f) is shown prior to (a, c, e) and following (b, d, f) LY2181308 treatment stained with H&E (1x in a and b; 20x in c and d) and survivin antibody (20x in e, f). Panel (g) shows the percent change of survivin mRNA and protein (nuclear and cytoplasmic) expression by IHC with medians and 95% confidence limits. Flow cytometric analysis of survivin expression by endobronchial NSCLC obtained from three patients by fiberoptic-guided bronchial brushing prior to and after LY2181308 (panel h) and change in high-survivin expressing cells (panel i).
Figure 2: Plasma and Tissue Pharmacokinetics Following 750 mg dose of LY2181308.

Plasma PK following loading and maintenance dosing (panel a). Observed values (open circles) are depicted with predicted exposures — median, .......... 5th and 95th percentiles).

Tissue levels of LY2181308 detected by IHC in two representative patients (b-e) before (b, d) and following loading dose (c, e) in tumor (10 out 11 patients showing a similar pattern) (b, c) and in stromal cells (5 of the 11 patients showing a similar pattern) (d, e). Tissue pharmacokinetics assessed by $[^{11}C]$LY2181308 uptake (AUC during 90 minute scan) scaled between the 0 and 140 ng*h mL$^{-1}$ window for a 1 mg dose: prior to first loading dose (f); and during the second half of the maintenance dose on day 15 (g). Inserts highlight change in uptake in specific area of mesothelioma over time.
Figure 3: Apoptosis Pathway Restoration and Pharmacodynamic Responses in Patients receiving 750 mg LY2181308. IHC detection of cleaved caspase 3 (panel a) and Ki67 (panel b) in tumor tissue obtained pre- and post-treatment with LY2181308. Percentage change from baseline are represented with medians and interquartile ranges. [$^{18}$F]FDG-PET images (40-60 min) uptake scaled between 0 and 17 g/mL (SUV): prior to first loading dose (c); and following the maintenance dose on day 22 (d). Inserts highlight change in uptake in mesothelioma tumor (same subject as figures 2 f & g).
Table 1. Baseline Patient and Disease Characteristics (N=40)

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<th>Category</th>
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<tr>
<td><strong>Sex</strong></td>
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<tr>
<td>Male</td>
<td>19 (47.5%)</td>
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<td>Female</td>
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<td>&lt;65</td>
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<td>&gt;65</td>
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<td>0</td>
<td>15 (37.5%)</td>
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<td>1</td>
<td>25 (62.5%)</td>
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<tr>
<td><strong>Pathological diagnosis (n)</strong></td>
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<tr>
<td>Gastrointestinal Tumors (including 7 colon, 1 rectal, 2 gastric, 1 esophageal, 1 pancreas cancer)</td>
<td>12 (30.0%)</td>
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<tr>
<td>Breast Cancer</td>
<td>8 (20.0%)</td>
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<tr>
<td>Melanoma</td>
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<tr>
<td>Lung Cancer</td>
<td>7 (17.5%)</td>
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<td>Other (3 sarcoma, 1 ovary, 1 head and neck, 1 unknown adenocarcinoma)</td>
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<td><strong>Prior therapy</strong></td>
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<tr>
<td>Radiotherapy</td>
<td>23 (57.5%)</td>
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<td>30 (75.0%)</td>
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<td>Chemotherapy</td>
<td>39 (97.5%)</td>
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Abbreviations: ECOG, Eastern Cooperative Oncology Group; NSCLC, non-small cell lung cancer
Table 2. Study Drug Related Adverse Events Occurring in 2 or More Patients (N=40 patients)

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<th>CTCAE Description</th>
<th>Maximum CTC Grade</th>
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<tr>
<td></td>
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<tr>
<td>Laboratory</td>
<td></td>
</tr>
<tr>
<td>PTT (Partial Thromboplastin Time)</td>
<td>28</td>
</tr>
<tr>
<td>Platelet Counts</td>
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<td>ALT/SGPT (serum glutamic pyruvic transaminase)</td>
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<tr>
<td>Metabolic/Laboratory – Other (including C-reactive Protein)</td>
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<td>Hemoglobin</td>
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<td>Lymphopenia</td>
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<td>AST/SGOT (serum glutamic oxaloacetic transaminase)</td>
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<td>Phosphate (hypophosphatemia)</td>
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<td>Leukocytes (total WBC)</td>
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<tr>
<td>Blood/Bone Marrow – Other (eosinophils etc)</td>
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<td>Non Laboratory</td>
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<tr>
<td>Fever (in the absence of neutropenia)</td>
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<tr>
<td>Nausea</td>
<td>7</td>
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<tr>
<td>Fatigue (asthenia, lethargy, malaise)</td>
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<td>Vomiting</td>
<td>6</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>2</td>
</tr>
<tr>
<td>Pain Neurology - Head/headache</td>
<td>2</td>
</tr>
<tr>
<td>Rigors/chills</td>
<td>2</td>
</tr>
<tr>
<td>Flu-like syndrome</td>
<td>1</td>
</tr>
<tr>
<td>Hypotension</td>
<td>1</td>
</tr>
<tr>
<td>Pain Musculoskeletal – Joint</td>
<td></td>
</tr>
<tr>
<td>Sweating (diaphoresis)</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 3. Plasma and Tissue Pharmacokinetics of LY2181308 at 750 mg

|                               | Predicted            | Observed            |
|                               | 1000 Simulations     | N=24                |
| Plasma Pharmacokinetic        |                       |                     |
| **AUC<sub>0-24</sub>** (ng.h/mL) | Mean (range)         | Mean (range)        |
|                               | 283725 (133922 – 582041) | 342794 (187344 – 603944) |
| **C<sub>max</sub>** ng/mL     | Mean (range)         |                     |
|                               | 65489 (42838 – 96897) | 69120 (39923 – 155514) |
| **C<sub>min</sub>** ng/mL     | Mean (range)         |                     |
|                               | 93.8 (63.9 – 131)    | 73.8 (36.8 - 135.9) |
| Tumor Tissue Pharmacokinetics |                       |                     |
| **Concentration** ng/mL       | Mean (range)         | ¹¹C-PET (n=4)       | ELISA (n=5) |
|                               | 33.2 (18.8 – 54.0)   | 32.5 (13.9-52.8)    | 22.4 (3.64 – 87.4) |
References


PET/CT) for tumor staging in solid tumors: comparison with CT and PET. J Clin Oncol 2004;22: 4357-68.


Fig 1

Research.
on April 20, 2017. © 2010 American Association for Cancerclincancerres.aacrjournals.org Downloaded from
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Pre

Post

Survivin (PE)

Counts

Pre-treatment

Post-treatment

% high-survivin staining

Patient 1

Patient 2

Patient 3

mRNA

Nucleus (IHC)

Cytoplasm (IHC)
Fig 2
Clinical Cancer Research

Tumor Survivin is Downregulated by the Antisense Oligonucleotide LY2181308: A Proof of Concept, First-in-Human Dose Study

Denis Talbot, Malcolm Ranson, Joanna Davies, et al.

Clin Cancer Res Published OnlineFirst November 1, 2010.

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