Case Series of Cancer Patients Treated With Galunisertib, a Transforming Growth Factor-Beta Receptor I Kinase Inhibitor in a First-in-Human Dose Study

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Abstract

Galunisertib (LY2157299 monohydrate) is a first-in-class small molecule inhibitor (SMI) of the transforming growth factor-beta (TGF-β) signaling pathway. Some adverse events were associated with galunisertib during the first-in-human dose (FHD) study. Among these adverse events (n = 11) were four cases of infection, two cases of thromboembolic events and two cases of thrombocytopenia. In one of the patients with thromboembolic events, an autopsy was also performed to examine possible changes of the aorta. No significant histopathologic changes were observed. Single cases of grade 2 diarrhea (only associated with drug intake), stroke (after resection for relapsed glioma), and pre-existing co-primary tumor (possible colorectal cancer) were observed. Because TGF-β plays an important role in tissue homeostasis and in immune response regulation, the present case series may serve as a future reference for adverse events observed in subsequent clinical trials with galunisertib.

Keywords: TGF-β Inhibitor; Galunisertib; First-in-human study; Glioma; Adverse events; Case reports

Introduction

Transforming growth factor-beta (TGF-β) signaling plays an important role in several diseases including cancer [1]; blocking TGF-β signaling is expected to result in anti-tumor activity. The small molecule inhibitor (SMI) of the TGF-β receptor I (RI) kinase galunisertib (LY2157299 monohydrate) shows cardiovascular toxicities in animals, such as changes at the

heart valves and aneurysms of the ascending aorta, similar to other SMI TGF-β inhibitors [2]. Also, long-term inhibition of TGF-β signaling may cause secondary cancers [3]. Because of these toxicity concerns for humans, clinical investigation of past SMI TGF-β inhibitors was not pursued. Using a predictive pharmacokinetic/pharmacodynamic model, a safe therapeutic window for galunisertib was proposed [4]. In a subsequent first-in-human dose (FHD) study, galunisertib was given 14 days on and 14 days off in a 28-day cycle within the predicted therapeutic window; no significant cardiovascular toxicities were observed [5]. The study consisted of three parts: part A, monotherapy of galunisertib and dose escalation; part B, combination of galunisertib with lomustine; part C, monotherapy to assess new formulations. Because galunisertib is the first-in-class SMI of TGF-β RI, the present case series of 11 patients may help to differentiate the adverse events of galunisertib from co-morbidities or other combined therapies (Table 1).

Case Reports

Part A (dose-escalation part and monotherapy of galunisertib)

Patient R30

This 48-year-old female patient with anaplastic astrocytoma was first treated with surgery and radiation. On the third relapse, she was started on galunisertib. After the first cycle, the patient showed a partial response (PR) which remained unchanged until progression a year later. Early in the treatment, the patient developed grade 2 diarrhea with abdominal cramping and gelatinous stool. The diarrhea was correlated with administration of galunisertib. The symptoms improved or resolved when galunisertib was not taken. The workup included examination for *Clostridium difficile* (negative), Salmonella/Shigella screen (negative), Giardia antigen (negative), *Campylobacter* culture (negative), ova and parasites (negative), fecal leukocytes (normal), hemoccults (negative), bacterial culture (negative), C-reactive protein (normal), and erythrocyte sedimentation rate (normal). Colonoscopy showed a normal colonic mucosa, no masses, no polyps or vascular abnormalities. Her total lymphocyte counts were low with a range of 720 - 1,420

Table 1. Patients With Important Responses and Adverse Events Observations in FHD Study

Patient ID	Diagnosis (WHO grade for glioma)	Main adverse event
Part A (galunisertib monotherapy)		
R30	Astrocytoma grade III	Diarrhea CTC grade 2
R12	Glioblastoma	Thromboembolic event
R15	Glioblastoma	Co-primary tumor of the gut
R23	Glioblastoma	Thromboembolic event
R28	Astrocytoma grade III	Stroke after re-resection
R35	Glioblastoma	Thrombocytopenia CTC grade 4
Part B (galunisertib + lomustine)		
R56	Glioblastoma	Salmonella infection
R63	Glioblastoma	Listeria infection
Part C (galunisertib monotherapy)		
R66	Glioblastoma	Legionella infection Fungal infection Herpes infection
R67	Glioblastoma	*E. coli* infection
R73	Glioblastoma	Thrombocytopenia CTC grade 3

(reference range: 1,100 - 4,800), but her CD4+ (37.8%) and CD8+ (37.3%) counts were within the lower normal range. The patient continued on treatment with galunisertib and no cause of the grade 2 diarrhea was found.

Patient R12

This 56-year-old male with a treatment-resistant glioblastoma began with galunisertib treatment after failing chemoradiation. Two months after starting on galunisertib, the patient had tumor progression, and was admitted to the hospital for dyspnea, hypoxia, tachycardia, and tachypnea. The ventilation perfusion scan showed pulmonary embolism, and an ultrasound confirmed right femoral-popliteal deep venous thrombosis. He was successfully treated with low molecular weight heparin (LMWH).

Patient R15

This 55-year-old male patient with glioblastoma multiforme was first surgically treated followed by chemoradiation with temozolomide (TMZ). This patient was on LMWH when he started on galunisertib and developed a new deep vein thrombosis, which was then successfully treated while on galunisertib. During the trial, two lung lesions were detected and thought to be inflammatory infiltrates. The patient received antibiotic therapy which did not improve his pulmonary condition. After the patient died, a limited autopsy of the pulmonary lesions identified them as an undifferentiated tumor. A positron emission tomography (PET) and computer tomography (CT) scan (Fig. 1) of the patient prior to his start on galunisertib showed a

rectal mural thickening, which improved during treatment with galunisertib. Concomitant with this observation, the PET marked the two lung lesions. Overall, this patient had likely a pre-existing and asymptomatic rectal neoplasia which had spread to the lung during the clinical trial or was already present in the lung at start of the treatment with galunisertib.

Patient R23

This 38-year-old male was originally diagnosed with a fibrillar astrocytoma and was treated with chemoradiation followed by adjuvant TMZ. About 4 years since his original diagnosis, a second surgery was performed and now a gemistocytic glioblastoma was diagnosed. He received additional TMZ and upon relapse started on galunisertib. He had no progression for over 1 year when his condition deteriorated and showed tumor progression. During the treatment with galunisertib, the patient had an episode of deep venous thrombosis and pulmonary emboli; these were treated while the patient continued on galunisertib. After the patient died due to tumor progression, a limited autopsy on the heart and ascending aorta was performed. The histopathologic examination (Fig. 2) showed a mild architectural alteration of the elastic fibers and mild mucinosis (Alcian blue staining), mild myxoid degeneration, and elastosis of the aortic valve. When compared with age-matched healthy aorta sections, these changes were not considered of significant pathological concern and within the variability of a general population. The findings were reviewed and confirmed by an independent pathologist (Eli Lilly and Company, data on file). The absences of major histopathologic changes were consistent with the unremarkable CT scans of the thorax assessing the ascending aorta and aortic arch during the trial, the echocardi-
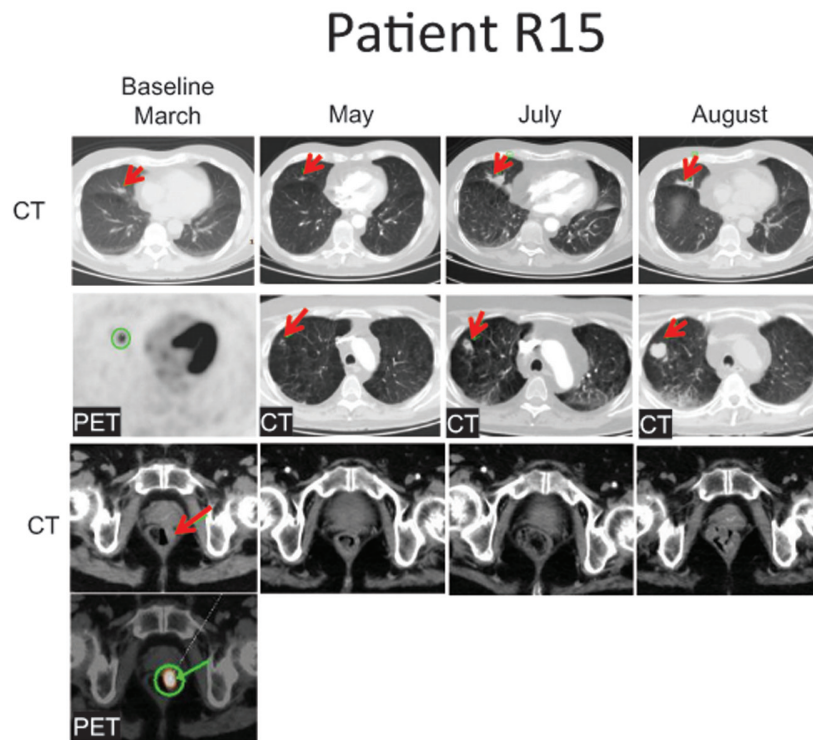
Patient R15

Figure 1. Computer tomography (CT) and positron emission tomography (PET) images of a patient (R15) with a co-primary tumor of the rectum (bottom two rows) with lung metastasis (top two rows). This patient was inadvertently enrolled in the study and retrospective evaluation showed a PET lesion in the rectal mucosa at baseline (green circle). A CT and PET scan also showed a lesion in the lung, which became gradually larger during the treatment with galunisertib (verified with CT scan).

ography/Doppler images and cardiac enzymes.

Patient R28

This 25-year-old male with anaplastic astrocytoma was treated with surgery followed by chemoradiation with TMZ. He was disease-free for 6 years before he had a first relapse which was treated with surgery and subsequent TMZ. On second relapse, he had a third surgery followed by treatment with galunisertib. With galunisertib a pre-existing fistula resolved and he was able to undergo a fourth resection, which was performed during the off-period of the first treatment cycle of galunisertib. In the immediate postoperative period, he had a stroke in the territory of the left medial cerebral artery, which was in the area of the surgery and hence it was considered not drug-related. Pre- and post-treatment tissue samples were examined for CD44 and inhibitor of DNA-binding protein (ID1) transcript levels by quantitative real-time polymerase chain reaction (qRT-PCR). Both CD44 and ID1 mRNA were reduced, suggesting on-target effect of galunisertib [6].

Patient R35

This 63-year-old man with glioblastoma was first treated with chemoradiation and TMZ followed sequentially by combination therapy of irinotecan/bevacizumab and monotherapy

bevacizumab. He tolerated galunisertib for the first cycle, but developed thrombocytopenia during the second cycle (cycle 2, day 14 grade 4 thrombocytopenia) without bleeding (Fig. 3). The patient was discontinued from the trial and his platelet counts improved gradually. On previous treatments with irinotecan/bevacizumab or monotherapy bevacizumab, his platelet counts also decreased (within the normal ranges) (Fig. 3). A few days after the thrombocytopenia, platelet aggregation assay was consistent with a primary inhibition of the hemostasis. The T3 was low with 54.8 ng/dL (normal range: 82.0 - 180.0 ng/dL) and results from the thyroid-stimulating hormone test were low with 0.01 mIU/L (normal range: 0.4 - 4.00 mIU/L). Also, $CD3^+CD4^+$ T cells were reduced at 0.250×10^9/L (normal range: $0.410 - 1.590 \times 10^9$/L). Results from a bone marrow biopsy excluded possible hematologic disorders. The serum autoimmune panel was negative. After 1 week of thrombocytopenia, his reticulocyte counts increased; after 3 weeks, his platelets were 81.1×10^9/L (grade 1). His platelet counts continued to normalize, but his tumor had progressed.

Part B (galunisertib combination with lomustine)

Patient R56

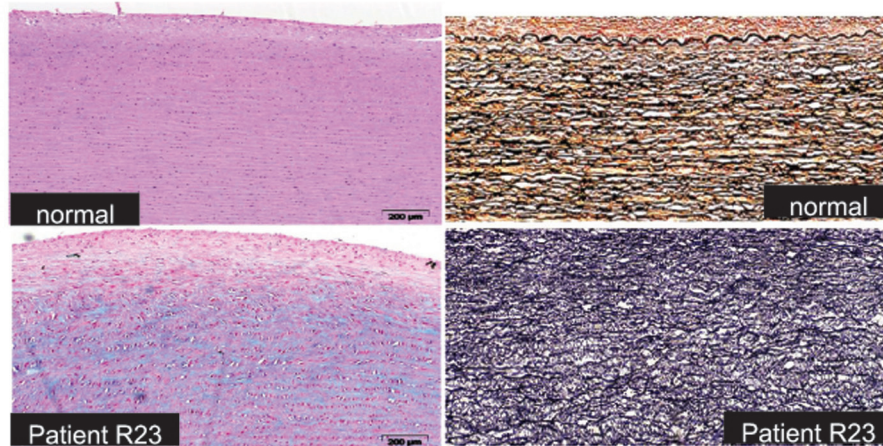This 44-year-old male patient with glioblastoma was first

Patient R23

Figure 2. Section of the aorta in a patient (R23) treated with galunisertib. From the patient's aorta different areas were stained with H&E staining and Alcian Blue (left two panels) and silver staining (right two panels).

treated with surgery followed by chemoradiation with TMZ. About 1 year later, there was a radiographic disease progression and the patient started on galunisertib and lomustine. Approximately 3 months after starting on galunisertib, the patient was hospitalized with gastroenteritis (grade 3), fever and hypotension (grade 4). He was successfully treated with fluid resuscitation, but a grade 4 neutropenia persisted. Subsequently,

a Salmonella infection was confirmed and with intravenous ceftriaxone the patient improved.

Patient R63

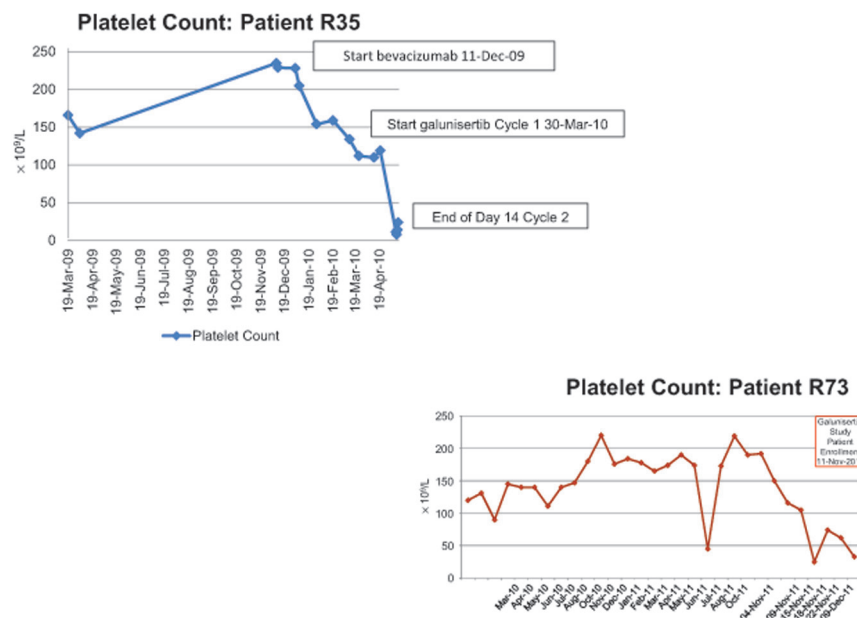This 59-year-old male with glioblastoma was first treated with

Figure 3. Thrombocytopenia in two patients (R35 and R73) treated with galunisertib. Both patients received prior bevacizumab and showed a reduction in platelet counts. However, both also showed a significant reduction in platelet counts after starting on galunisertib.

surgery followed by chemoradiation with TMZ. After his first relapse, he underwent surgery and later started on the combination of galunisertib and lomustine. Approximately 1 month later, the patient developed severe diarrhea and fever (grade 3) and both study medications were held. Abnormal biochemistry included creatinine of 0.63 mg/dL, sodium 132 mEq/L, total protein 5.5 g/dL, albumin 3.3 g/dL, calcium 8 mg/L, LDH 641 U/L, and C-reactive protein 1 mg/dL. Hematology abnormalities included leukocytes $2.12 \times 10^3/\mu L$, hematocrit 37.1%, platelets $96 \times 10^3/\mu L$, lymphocytes $0.33 \times 10^3/\mu L$, monocytes $0.16 \times 10^3/\mu L$, and neutrophils $1.60 \times 10^3/\mu L$. Listeria monocytogenes were isolated from blood cultures and antibiotic treatment with nystatin, ciprofloxacin, ampicillin sodium, sulfamethoxazole, trimethoprim, and gentamicin sulphate was started. The infection resolved within 2 weeks and he continued on galunisertib.

Part C (monotherapy; relative bioequivalent study)

Patient R66

This 52-year-old female with glioblastoma underwent surgery followed by chemoradiation with TMZ, but due to rapid progression, she first received an investigational agent together with TMZ and bevacizumab for six cycles. She then relapsed and was started on galunisertib. Two weeks after starting on galunisertib, she was diagnosed with bilateral pneumonia, neutropenia (grade 4), oral herpes infection, and fungal mucositis; grade 2 thrombocytopenia was also diagnosed. The urinalysis was positive for Legionella antigen. Culture of exudates from a skin blister showed herpes simplex virus 1 and herpes simplex virus 2 by qRT-PCR, varicella virus by qRT-PCR. Culture of exudate from oral cavity showed herpes simplex virus 1 by qRT-PCR, herpes simplex virus 2 by qRT-PCR. Antibiotic treatment was started with meropenem, amikacin sulphate and azithromycin. She was also started on fluconazole and acyclovir. Despite these interventions, she progressed to respiratory insufficiency and succumbed.

Patient R67

This 51-year-old female with glioblastoma received chemoradiation with TMZ. On relapse, she was treated with a novel agent and on second relapse with galunisertib. During her treatment with galunisertib, she had three adverse events: 1) pulmonary embolism with no clinical symptoms, treated with LMWH during cycle 1; 2) diarrhea (grade 3) without blood in the stools, treated with intravenous fluids (laboratory parameters were normal, stool culture was negative) resolving her diarrhea and galunisertib administration was resumed; 3) a second episode of afebrile diarrhea (grade 3) without blood in the stool after completing the 14-day treatment of cycle 2 (tests were negative for *Clostridium difficile*, Salmonella, Shigella, Yersinia, and Campylobacter). Cytomegalovirus and Epstein-Barr virus serologies were positive for IgG. The lymphocyte immunophenotype was normal. The Ig subtypes showed a

slight decrease of IgG and IgM. The levels of antinuclear and antiparietal cell antibodies were low, and autoimmune diarrhea was ruled out. A colonoscopy found normal colon mucosa. The diarrhea resolved with stringent diet and loperamide. The patient remained hospitalized because the tumor was progressing. In the hospital, she developed fever and blood culture was positive for *E. coli*. She was treated with antibiotics and recovered from the infection.

Patient R73

This 51-year-old male with glioblastoma received neoadjuvant treatment with bevacizumab and TMZ followed by radiotherapy with bevacizumab and TMZ. Upon first and second relapse, he was treated with irinotecan and bevacizumab; on third relapse, he was treated with lomustine and bevacizumab. On the fourth relapse, he began treatment with galunisertib. While on previous anti-cancer treatments, he had transient thrombocytopenia (grade 1) and after starting on galunisertib he had thrombocytopenia (grade 3, platelet count $25.5 \times 10^9/L$) with an episode of rectal hemorrhage (grade 1). He was treated with antibiotics (amoxicillin + clavulanic acid followed by levofloxacin) because of a skin infection caused by *Staphylococcus aureus*. As treatment with galunisertib continued, platelet counts dropped further (grade 3) and he experienced two episodes of gastrointestinal hemorrhage and one episode of epistaxis (grade 1). Serum autoantibodies (e.g., rheumatoid factor, anti-mitochondrial, anti-muscle, anti-cardiolipin, and anti-nuclear antibodies) were negative. Serology tests for viral hepatitis, human immunodeficiency virus, cytomegalovirus, and Epstein-Barr virus were negative. The immunoglobulin profile was slightly decreased, and complement levels (C3 and C4) were normal. A blood smear displayed no aggregates, and the autoimmune thrombocytopenia was also negative. The blood marrow biopsy found a hypocellular marrow without neoplastic cells or marrow fibrosis. About 1.5 months later and after severe infections, he had a worsening of neurological symptoms with tumor progression.

Discussion

Patients with glioblastoma suffer from several co-existing conditions, such as venous thromboembolism (up to 70%) and "other" conditions (17%), including vertigo, hemorrhage, and infection [7, 8]. Consistent with this observation, two patients (R12 and R23) had thromboembolic events; these resolved while patients were treated for the events and received galunisertib. Hence, the observed severe thromboembolic events in this study did not exceed the known event rate in glioblastoma patients.

Severe thrombocytopenia was observed in two cases (R35 and R73) (Fig. 3); in both patients, the bone marrow was hypocellular. In animal toxicology studies and in human bone marrow cultures, galunisertib had no direct bone marrow toxicity (data on file, Eli Lilly and Company). Hence, other causes for these findings were considered. In patient R35, CD3$^+$CD4$^+$

counts were reduced at time of the event and prior bevacizumab was associated with reduction in platelet counts, an adverse event previously reported [9]. Both of these findings imply a possible secondary immune thrombocytopenia (ITP) [10]. The second patient (R73) with thrombocytopenia had also previous platelet reductions during bevacizumab treatment (grade 1). However, during the galunisertib treatment there were no changes in T cell subsets and the co-existing infection may have influenced the bone marrow regeneration. In chronic secondary ITP, T regulatory cells are required to suppress the immune activity directed against platelets [11]. Since T regulatory cells produce TGF-β and galunisertib may block this TGF-β signaling, it is possible that the immunological control was removed in both of these patients. Ipilimumab is also a drug that targets the T regulatory cells and its toxicity profile includes occasional thrombocytopenia of unclear origin [12]. However, the bone marrow biopsy findings are not entirely consistent with a secondary ITP; hence, the cause of both cases remains unclear and not comparable to ipilimumab.

The diarrhea in patient R30 also suggests a possible or subtle immune dysregulation. Although her total lymphocyte counts were low, her subsets were in the normal range. In contrast to galunisertib, ipilimumab causes a diarrhea rate of about 30%, which requires a transient stop in treatment or a course of steroids [13].

Given the concern that galunisertib may cause immune dysregulation, response to infection was reviewed. In contrast to part A, all major infections occurred in part B and part C. In part B, patients had reduced lymphocyte counts likely due to the lomustine treatment. The three patients from parts B and C (R56, R63 and R67) were successfully treated with antibiotics while on galunisertib. In part C, however, there was a fourth patient who had a profound immune deficiency (R73) with thrombocytopenia. This patient had galunisertib monotherapy and did not improve with antibiotic and supportive treatment. Given the severity of this case, it remains unclear whether in such patients galunisertib has a negative impact on immune responses to sepsis or infection. Other possible toxicity concerns that are associated with TGF-β inhibitors were not observed, such as cardiotoxicities or induction of secondary cancers. The autopsy case of patient R23 implied that galunisertib had no significant functional change on the structure of the aorta (Fig. 2). Also, patient R15 with a co-primary tumor was inadvertently enrolled as the retrospective evaluation of his PET scans suggested (Fig. 1). None of the other patients treated with galunisertib showed pre-neoplastic lesions as observed for the monoclonal antibody fresolimumab [14].

In summary, the present case series will help guide future adverse event findings in the subsequent clinical trials with galunisertib and thus help with recognizing potential drug-associated adverse events.

Conflict of Interest

Michael Carducci, Juan M. Sepulveda-Sanchez, Analia Azaro, and Matthias Holdhoff have no conflict of interest. Jordi Rodon served on advisory board meetings of Eli Lilly and Company and received compensation. Drs. Lahn, Gueorguieva, Pillay, Desaiah, Ms. Cleverly are employees of Eli Lilly and Company, Indianapolis, IN, USA and may hold company stocks.

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