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PHASE I STUDIES

A phase I study of the farnesyltransferase inhibitor Tipifarnib in combination with the epidermal growth factor tyrosine kinase inhibitor Erlotinib in patients with advanced solid tumors

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Summary

Introduction Based on preclinical cytotoxic synergy between tipifarnib and erlotinib, a phase I study of this combination was conducted in patients with advanced solid tumors to evaluate safety, tolerability, maximum tolerated dose (MTD) and preliminary evidence of efficacy. *Methods* Patient enrollment followed the traditional "3 + 3" dose escalation scheme, through 4 dose levels, ranging from tipifarnib 200 mg twice daily plus erlotinib 75 mg once daily to tipifarnib 300 mg twice daily plus erlotinib 150 mg once daily. After the MTD of the combination was identified, 12 additional patients were treated to better define the pharmacokinetics and pharmacodynamics of these agents. *Results* A total of 27 patients were enrolled in the study (dose escalation, 15; dose expansion, 12). Dose limiting toxicity was seen in one patient at dose level 4 (grade 3 diarrhea). The MTD was reached at erlotinib 150 mg once daily combined with tipifarnib 300 mg twice daily. The most common side effects of the combination of all grades were diarrhea (85.2%), fatigue (77.8%), rash (70.4%), and anorexia (59.3%). Overall, 2 patients (7.4%; with liver cancer and melanoma, respectively) had partial responses, 10 (37%) had stable disease, 11 had progressive disease (40.7%) and 4 stopped treatment prematurely for assessment. *Conclusion* The combination of tipifarnib and erlotinib was well tolerated. Erlotinib 150 mg once daily for 28 days combined with tipifarnib 300 mg twice daily for 21 days was identified as the recommended phase 2 dose. Tipifarnib is currently being evaluated in *HRAS* mutant tumors, providing a potential opportunity to further test this combination.

Keywords Tipifarnib · Erlotinib · Phase I · Farnesyltransferase inhibitor · Solid tumors · RAS

Introduction

With *RAS* oncogenes being the first human oncogenes discovered in human cancers more than 35 years ago, the RAS/MAPK (mitogen-activated protein kinase) pathway is one of the best studied signaling pathways in oncology [1–4]. RAS proteins are small GTPases that play crucial roles in biochemical pathways responsible for a variety of functions such as cellular growth, proliferation, cytoskeletal alterations, cell migration, and even cell death. They do so by responding to multiple receptor tyrosine kinases (e.g., epidermal growth factor receptor) and regulating numerous cascades with various downstream targets (e.g., Raf/Mek/MAPK) [5, 6]. There are three *RAS* genes (HRAS, NRAS, KRAS), and mutations in these genes have been shown to play a role in the development in about 30% of all human tumors, including colorectal, bladder, and lung cancers, with KRAS being the predominant culprit [3, 7–10].

Farnesyltransferase (FT) is a zinc metalloenzyme that plays an essential role in the post-translational modification of RAS proteins by adding a 15-carbon isoprenyl farnesyl moiety to the carboxy terminus of proteins containing a "CAAX" motif, thereby allowing the proteins to properly associate with the plasma membrane [11–13]. FT inhibitors such as tipifarnib were designed to inhibit the farnesylation of a variety of proteins, including the RAS isoforms. These inhibitors have been shown to have anticancer effect in preclinical models [14–16] and numerous clinical trials [17–20]. In light of the observation that FT inhibitors are active in neoplasms without RAD mutations [17], it as has also been suggested that FT inhibition

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can lead to antitumor activity through substrates other than RAS, such as the G protein RHOB, a cytoskeletal regulator responsible for trafficking of cell surface receptors [21] and the phosphoinositide 3-OH kinase (PI3K)/AKT pathway [22].

Tipifarnib has undergone multiple phase I trials demonstrating its antineoplastic effects in both solid tumors [18, 19, 23, 24] and leukemia [17]. The toxicities noted included myelosuppression with leukocytopenia and neutropenia, neurotoxicity with ataxia, confusion and neuropathy, nausea, vomiting, and fatigue [17–19]. The drug was shown to inhibit FT at 300 mg bid and accumulated in the bone marrow in a dose-dependent fashion, with large increases in marrow drug levels beginning at 600 mg bid. Based on these studies, the recommended phase II dose of tipifarnib was 500 mg twice daily for 5 days every 14 days or 300 mg twice daily for ≥21 days in solid tumors and 600 mg bid for 21 days in acute leukemia.

While the results of phase II studies for single agent tipifarnib in refractory lymphomas [20] and breast cancer were promising [25], other phase II trials did not demonstrate significant efficacy of single agent tipifarnib in metastatic pancreatic cancer [26], hormone-refractory prostate cancer [27], melanoma [28], small-cell lung cancer [29], and non-small-cell lung cancer (NSCLC) [30]. A phase III trial in advanced colorectal cancer also demonstrated that single agent tipifarnib has an acceptable toxicity profile but did not improve overall survival [31]. Tipifarnib is currently undergoing further testing in *HRAS*-mutant tumors such as thyroid cancer and uroepithelial cancer as well as in selected myeloid malignancies [32].

Tipifarnib was further studied in combination with other agents such as gemcitabine, cisplatin, docetaxel, temozolomide and etoposide in patients with advanced solid malignancies and leukemias. The results indicated that tipifarnib did not alter the pharmacokinetics of these agents, and the combinations were well tolerated but efficacy was modest at best [33–37].

Epidermal growth factor receptor (EGFR) and its ligands EGF and TGF-alpha play key roles in tumor cell proliferation, survival, adhesion, motility, invasion, and angiogenesis [38, 39]. EGFR inhibitors have shown efficacy in non-small cell lung cancers harboring activating mutations in the kinase domain of *EGFR* and in KRAS wild-type colorectal cancer.

Erlotinib is an orally active EGFR tyrosine kinase inhibitor in clinical use for *EGFR* mutant NSCLC. This agent also demonstrates moderate efficacy when combined with gemcitabine in metastatic pancreatic cancer [40].

Preclinical data suggest that the RAS/MAPK pathway is involved in the activities of both tipifarnib and erlotinib [41, 42]. We therefore generated preclinical data on the synergistic toxicity between erlotinib and tipifarnib in human cultured cell lines. Based on our findings, the current study was undertaken to identify the maximum tolerated dose of tipifarnib in combination with erlotinib, describe the toxicity profile of this combination, and evaluate the preliminary anticancer efficacy of the combination.

Methods

Study design

This was a phase I study of the combination of tipifarnib and erlotinib, with patients enrolled in a traditional "3 + 3" dose escalation scheme. There were 4 dose levels (Table 1), ranging from tipifarnib 200 mg twice daily plus erlotinib 75 mg once daily up to tipifarnib 300 mg twice daily plus erlotinib 150 mg once daily. Three patients were treated at each dose level for 28-day cycles and observed for a minimum of 4 weeks before new patients were treated. Tipifarnib was administered on Days 1–21 with one week off, while erlotinib was administered continuously throughout the 28 days. Patients were assigned their dose level by the Mayo Clinic Cancer Center (MCCC) Randomization Center and continued on treatment until they experienced unacceptable side effects, had disease progression, or withdrew consent. Doses were not escalated in any individual patient.

After the MTD of the combination was identified, 12 additional patients were treated to better define the pharmacokinetics and pharmacodynamics of these agents.

Patient selection

Patients with confirmed locally advanced, inoperable or metastatic solid tumors were eligible for the study. Other inclusion criteria included age ≥ 18 years; Eastern Cooperative Oncology Group (ECOG) performance status 0–1; adequate liver (total bilirubin ≤2 mg/dL, ALT and AST ≤2.5× ULN), renal (creatinine <1.5× ULN), and bone marrow (absolute neutrophil count $\geq 1.5 \times 10^9$/L; platelets $\geq 100 \times 10^9$/L; Hgb ≥9 g/dL) functions. Patients were excluded if there was a known standard therapy for the patient's disease that was potentially curative or definitely capable of extending life

Table 1 Dose escalation plan

Dose level	Erlotinib (mg, QD)	Tipifarnib (mg, BID)
-1	50	100
0	50	200
1*	75*	200*
2	100	200
3	100	300
4	150	300

*Starting dose

expectancy. Other exclusion criteria included an ECOG performance status (PS) of 2, 3, or 4; uncontrolled infection; received chemotherapy, immunotherapy, hormonal therapy, or radiation therapy within 4 weeks prior to study entry; and prior treatment with EGFR targeting therapies. Concurrent intake of CYP3A4-inducing agents was not allowed.

Dose-limiting toxicity (DLT) and maximum tolerated dose (MTD)

The severity of adverse events was evaluated according to the National Cancer Institute Common Terminology Criteria of Adverse Events (CTCAE) version 4 [43]. Treatment related DLT was defined by the occurrence of any of the defined toxicities definitely or probably related to study drug(s) within the first cycle (28 days) of treatment including: Grade 4 ANC for ≥5 days or Grade 4 thrombocytopenia of any duration, serum creatinine ≥2 times baseline or ≥ 2 times ULN, Grade ≥ 3 vomiting despite maximal anti-emetic support, Grade ≥ 3 diarrhea despite maximal antidiarrheal support, and ≥ Grade 3 rash despite adequate therapy. The MTD was defined as the dose level below the lowest dose that induced DLT in at least one-third of patients (at least 2 of a maximum of 6 new patients).

Patient evaluation

Patients were monitored continuously throughout the study. A complete history and physical examination was done prior to each cycle, and CBC and blood chemistries were done prior to each cycle and weekly during each cycle for the first two cycles. Imaging such as a CT scan or MRI was done at baseline and at 8- to 12-week intervals for the evaluation of efficacy using the Modified Response Evaluation Criteria in Solid Tumors (RECIST) criteria [44]. Patients were followed for a maximum of 3 months after completing treatment.

Pharmacodynamic studies

For pharmacodynamic studies to evaluate the determinants of response and toxicity to this combination, patients treated at the MTD underwent two tumor biopsies: the first was on or before day 1, and the second was on day 21 of cycle 1.

Materials

Tipifarnib for tissue culture was provided by Johnson & Johnson (New Brunswick, NJ, USA). Electrophoresis grade chemicals for SDS-polyacrylamide gel electrophoresis were from Bio-Rad (Hercules, CA). Antibodies to the indicated antigens were purchased as follows: Murine anti-HDJ-2 from Neomarkers (Freemont, CA); rabbit polyclonal antibodies to phospho-Ser$^{240/244}$-S6, phospho-Thr^{202}/Tyr^{204}-ERK1/2, phospho-Ser^{473}-AKT, total ERK1/2 and total AKT from Cell Signaling Technology (Beverly, MA). Rabbit polyclonal antibodies to prelamin A [45] and chicken antibodies to total lamin A/C [46] were raised as described. Murine monoclonal antibody to HSP90β was a kind gift from David Toft (Mayo Clinic, Rochester, MN).

Immunoblotting

Biopsy samples harvested prior to day 1 therapy and on day 21 approximately 12 h after the last dose of tipifarnib were snap frozen at the point of acquisition and stored on liquid nitrogen until analyzed. Samples were rapidly thawed and sonicated in buffered 6 M guanidine hydrochloride under reducing conditions, treated with iodoacetamide, and dialyzed sequentially into 4 M urea and 0.1% SDS as previously described [47, 48]. Aliquots containing 50 μg of protein (assayed by the bicinchoninic acid method) were separated by SDS-polyacrylamide gel electrophoresis, transferred to nitrocellulose, and probed with antibodies as previously described [49]. HL-60 human acute myelogenous leukemia cells treated with diluent for 1 μM tipifarnib for 24 h served as a negative and positive control for tipifarnib-induced changes in signaling [50].

Results

Patient characteristics

A total of 27 patients, median age 59, with advanced solid tumors were enrolled in the study, with 15 in the dose escalation portion and 12 in the dose expansion. Patient demographics and pretreatment characteristics are summarized in Table 2. Thirty-three percent were male, all had an ECOG performance status of 0–1, and all were Caucasian. All patients had undergone prior surgeries, and the majority of them (77.8%) had been previously treated with chemotherapy.

DLT and MTD

All 15 patients in the dose escalation portion were evaluable for DLT. Nine patients were treated at dose level 1–3 (3 patients per dose level) with no DLT observed. Six patients were enrolled at dose level 4, and DLT was seen in one patient at dose level 4 (grade 3 diarrhea). No grade 5 toxicity was seen. The MTD was defined as 150 mg of erlotinib once daily for 28 days combined with 300 mg of tipifarnib twice daily on days 1–21 (dose level 4). These doses represented the full single agent doses of both drugs. Twelve patients were further treated at the MTD level in the dose expansion group. Two of these patients developed grade 3 fatigue, one developed grade 3 anxiety and 1 had an episode of syncope in Cycle 1.

Table 2 Patient characteristics

	Overall ($N = 27$)	Cohort I ($N = 15$)	Cohort II ($N = 12$)
Age, median(range)	59 (25,80)	47 (25,80)	65 (46,75)
Gender			
Female	18 (66.7%)	11 (73.3%)	7 (58.3%)
Male	9 (33.3%)	4 (26.7%)	5 (41.7%)
Race			
White	27 (100%)	15 (100%)	12 (100%)
Performance score			
0	16 (59.3%)	11 (73.3%)	5 (41.7%)
1	11 (40.7%)	4 (26.7%)	7 (58.3%)
Prior treatments			
Chemotherapy	21 (77.8%)	10 (66.7%)	11 (91.7%)
Radiation therapy	14 (51.9%)	6 (40%)	8 (66.7%)
Surgery	27 (100%)	15 (100%)	12 (100%)

Safety and tolerability

All 27 patients were evaluable for safety analysis (Table 3). The combination of tipifarnib and erlotinib was well tolerated. A summary of the toxicities is provided in Table 3**.** The most common side effects (of all grades) of the combination were diarrhea (85.2%), fatigue (77.8%), rash (70.4%), anorexia (59.3%), nausea (51.9%), anemia (37%), and pruritus (33.3%). The vast majority of these toxicities were Grade 1–2 (Table 3).

Preliminary antitumor activity

The tumor responses were assessed according to the RECIST criteria. Overall, 2 patients (7.4%) had partial responses, 10 patients (37%) had stable disease, 11 had progressive disease (40.7%), and 4 patients (14.8%) went off treatment before their responses could be assessed (one due to adverse events, and three refused further participation) (Table 4).

Out of the 10 patients who had stable disease as their best response, one patient had stable disease for 41 cycles, one patient had stable disease for 35 cycles, one patient had stable disease for 6 cycles, one patient had stable disease for 3 cycles, and six patients had stable disease for 1 cycle.

Out of the 2 patients who had partial response as their best response, one patient with liver cancer had PR for 14 cycles and one patient with melanoma had PR for 5 cycles.

Pharmacodynamic assessment

To assess the signaling changes induced by the treatment, paired biopsies (one harvested before the start of therapy and one on day 21 of cycle 1) were subjected to immunoblotting for changes in signaling pathways potentially affected by the two agents. Results obtained in 5 samples are shown in Fig. 1. Inhibition of farnesylation was readily apparent in 5 of 6 samples, including samples from patients 1, 2, 4, and 5 in Fig. 1, as shown by increased unprocessed HDJ-2 and increased prelamin A, two species that are detected when farnesylation-dependent processing is impaired [45, 51]. Moreover, there was evidence of inhibition of downstream signaling through the PI3K/AKT/mTOR pathway, as manifested by increased phosphorylation of AKT Ser^{473} in samples patients 3 and 5.

In contrast, we were unable to detect phospho-EGFR or total EGFR in these samples despite numerous attempts with multiple different reagents. Moreover, there was no inhibition of signaling through RAF/MEK/ERK pathways, as would be expected if EGFR had been inhibited. Accordingly, we saw good evidence for inhibition of protein farnesylation but little evidence for extensive MAPK pathway inhibition at exposures of the combination achieved in the clinical setting.

Discussion

While single-agent activities of both tipifarnib and erlotinib have been reported in several prior single-agent studies [18, 23, 52, 53], this is the first and as of now the only phase I trial combining these two agents. Both of these agents target similar pathways in cellular signaling, particularly the RAS/MAPK and the PI3-K/AKT pathways, albeit at different levels [5, 14, 54, 55]. This may explain the synergistic anti-tumor activity seen in preclinical studies.

From the results of our study, this combination is safe. The recommended dosing for future studies is erlotinib 150 mg daily combined with tipifarnib 300 mg twice daily for 21 out of every 28 days. These represent the full single-agent doses of each drug. In spite of these full doses, toxicity was modest. The most common toxicities were gastro-intestinal (diarrhea, anorexia, nausea),

Table 3 Summary of toxicities

Toxicity	All (%) N = 27	Grade 1–2 (%)	Grade 3–4 (%)
General			
Fatigue	21 (77.8)	18 (66.7)	3 (11.1)
Weight loss	3 (11.1)	3 (11.1)	0
Edema	1 (3.7)	1 (3.7)	0
Gastrointestinal			
Diarrhea	23 (85.2)	20 (74.1)	3 (11.1)
Anorexia	16 (59.3)	16 (59.3)	0
Nausea	14 (51.9)	13 (48.1)	1 (3.7)
Vomiting	7 (25.9)	7 (25.9)	0
Skin			
Rash	19 (70.4)	19 (70.4)	0
Pruritis	9 (33.3)	8 (29.6)	1 (3.7)
Dry Skin	8 (29.6)	8 (29.6)	0
Alopecia	4 (14.8)	4 (14.8)	0
Renal			
Elevated creatinine	2 (7.4)	2 (7.4)	0
Hematological			
Anemia	10 (37)	7 (25.9)	3 (11.1)
Neutropenia	4 (14.8)	2 (7.4)	2 (7.4)
Thrombocytopenia	10 (37)	10 (37)	0
Leukopenia	6 (22.2)	4 (14.8)	2 (7.4)
Liver			
AST elevation	10 (37)	9 (33.3)	1 (3.7)
Bilirubin	7 (25.9)	6 (22.2)	1 (3.7)
Electrolytes			
Hypocalcemia	5 (18.5)	5 (18.5)	0
Hyperglycemia	6 (22.2)	6 (22.2)	0
Hypokalemia	5 (18.5)	4 (14.8)	1 (3.7)
Hyponatremia	5 (18.5)	4 (14.8)	1 (3.7)
Neurological			
Headache	4 (14.8)	4 (14.8)	0
Dizziness	3 (11.1)	3 (11.1)	0
Neurosensory complaints	4 (14.8)	4 (14.)	0
Dry eye	3 (11.1)	3 (11.1)	0
Ataxia	1 (3.7)	1 (3.7)	0
Confusion	1 (3.7)	1 (3.7)	0
Anxiety	1 (3.7)	0	1 (3.7)

Table 4 Tumor responses

	Overall (N = 27)	Cohort I (N = 15)	Cohort II (N = 12)
Best response			
Partial response	2 (7.4%)	0	2 (16.7%)
Stable disease	10 (37%)	9 (60%)	1 (8.3%)
Progressive disease	11 (40.7%)	6 (40%)	5 (41.7%)
Too early to assess*	4 (14.8%)	0	4 (33.3%)

*One patient discontinued due to adverse events, three refused further participation

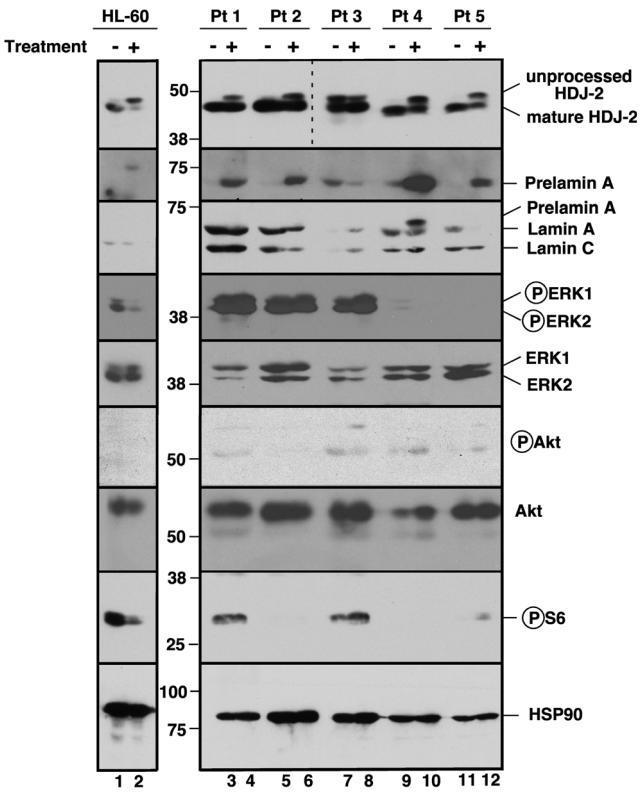

Fig. 1 Pharmacodynamic assessment. Aliquots containing paired biopsies from the same patient obtained before (−) or during (+) treatment with tipifarnib and erlotinib (lanes 3–12) were subjected to immunoblotting for the indicated antigens. HL-60 cells treated with diluent (−) or tipifarnib (+) were included on the same blot as a positive control for the tipifarnib-induced inhibition of HDJ-2 and prelamin A processing as well as inhibition of downstream signaling (lanes 1, 2). Clinical responses of the patients are as follows – Pt 1 = PD at cycle 2, Pt 2 = PR at cycle 25, Pt 3 = PD at cycle 8, Pt 4 = PD at cycle 1, Pt 5 = PD at cycle 1

dermatological (rash, pruritis), hematological (anemia, neutropenia) and fatigue. One patient in the dose expansion cohort discontinued treatment due to toxicity.

The side effects seen in this study are similar to those of erlotinib and tipifarnib administered as single-agents. Thus, the toxicity of these agents was not exacerbated by the combination. Two patients, one with liver cancer and one with melanoma, had partial responses, and 10 of the total 27 patients had durable stable disease.

In selected patients at the recommended phase II dose, pharmacodynamics effects were documented. Using HDJ-2 as the biomarker, inhibition of farnesylation was demonstrated in tumor samples from 5 of 6 patients. In addition, inhibition of downstream signaling through the PI3K/AKT/mTOR pathway was documented by the increased phosphorylation of AKT Ser^{473} in some tumor samples. However, neither phospho-EGFR nor total EGFR could be detected in these samples, and there was no inhibition of signaling through RAF/MEK/ERK pathways, as might be expected if EGFR had been inhibited. Thus, taken together, the biomarker studies demonstrated inhibition of farnesylation but could not document EGFR inhibition in the tumor samples tested. It is possible, of course, that EGFR levels in these lung cancers might have been below the limit of detection by immunoblotting and that RAF/MEK/ERK pathway activation was driven by alternative receptor tyrosine kinases. Consistent with these possibilities, the response rate to single agent erlotinib is reportedly higher in non-small cell lung cancers with higher EGFR expression [56]. While the correlative studies were performed in only a subset of patients enrolled in the present trial, the low EGFR levels and seeming lack of dependence of RAF/MEK/ERK signaling on EGFR might explain, at least in part, the lack of a more robust clinical activity in this study.

Tipifarnib inhibits farnesyltransferase, which is an enzyme that catalyzes the addition of a 15-carbon farnesyl group to a small group of hydrophilic cellular polypeptides, including the heat shock protein HDJ-2 and certain members of the RAS, RHO and RHEB families. The earlier focus of studies with tipifarnib was in *KRAS* mutant tumors. It became clear, however, that KRAS could be alternatively prenylated through geranylgenerylation when farnesylation was inhibited [57]. This has been seen in part as the reason for the lack of robust activity of this compound. In recent years, mutant *HRAS* has become identified as a predictive biomarker of the efficacy of tipifarnib. There are, therefore, ongoing studies of tipifarnib in *HRAS* mutant squamous cell lung (NCT03496766), head and neck (NCT02383927) and urothelial tumors (NCT02535650). Thus, there may be some utility in testing tipifarnib in combination with erlotinib or another EGFR inhibitor in some of these tumors in future.

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Compliance with ethical standards

Conflicts of interests MG is consultant for Pfizer Inc., Eli Lilly and Company, and Novartis. KJ, JM, JA, JY, JR, V-SL, SHK, and AA have no conflict of interest to report.

Ethical approval All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors.

Informed consent Informed consent was obtained from all individual participants included in the study.

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