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Neratinib Alone or in Combination with Immune Checkpoint Inhibitors with or without Mammalian Target of Rapamycin Inhibitors in Patients with Fibrolamellar Carcinoma

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Keywords
Neratinib · Fibrolamellar carcinoma · SUMMIT phase 2 basket study · Pan-HER kinase inhibition

Abstract

Introduction: Fibrolamellar carcinoma (FLC) displays upregulation of several oncogenes, including *HER2*, and multiple immune-suppressive mechanisms. We investigated the efficacy and safety of the pan-HER tyrosine kinase inhibitor neratinib as monotherapy (SUMMIT phase 2 basket study) or with immune checkpoint and/or mammalian target of rapamycin (mTOR) inhibitors (compassionate-use program) in patients with FLC. ***Methods:*** Patients received neratinib 240 mg/day orally in SUMMIT, or as doublet or triplet combinations with pembrolizumab 2 mg/kg intravenously every 3 weeks, nivolumab 240 mg intravenously every 2 weeks, everolimus 7.5 mg/day orally, or sunitinib 37.5 mg/day orally under compassionate use. The primary endpoint in SUMMIT was objective response rate; safety was a secondary endpoint. ***Results:*** Fifteen patients with FLC received neratinib monotherapy in SUMMIT. The objective response rate was 5% (95% confidence interval [CI]: 0–21.8) and the disease control rate was 13.3% (95% CI: 1.7–40.5). Upon

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progression, five had added immune checkpoint inhibitors with or without everolimus or sunitinib. Two additional patients received neratinib-based combinations outside of SUMMIT, for a total of 17 neratinib-treated patients. One patient who received neratinib plus pembrolizumab had a confirmed partial response, one treated with neratinib plus everolimus had stable disease lasting 6 months, and one who received neratinib plus pembrolizumab plus sunitinib had stable disease lasting 16 months. Grade 3/4 adverse events with neratinib monotherapy occurred in 10 (66.7%)/2 (13.3%) patients, respectively. Grade 3 adverse events with neratinib-based combinations were hyperglycemia (n = 1; neratinib plus pembrolizumab), hepatic failure, and anaphylaxis (n = 1 each, neratinib plus pembrolizumab plus everolimus). There were no grade 4 adverse events with combination therapy. ***Conclusion:*** In patients with FLC, single-agent neratinib had limited efficacy, but clinical benefit was observed with neratinib in combination with immunotherapy and/or mTOR-targeted agents.

Introduction

Fibrolamellar carcinoma (FLC) is an ultra-rare cancer that is distinct from classical hepatocellular carcinoma with respect to epidemiology, etiology, and prognosis [1, 2]. This tumor typically affects younger adults and adolescents aged between 10 and 30 years with no history of cirrhosis or hepatic dysfunction [3]. Effective therapeutic options are limited. Surgery remains the mainstay of therapy; however, 20–30% of patients have unresectable disease at presentation [4, 5], and over 50% of patients who are candidates for surgery will have disease recurrence and ultimately die of their cancer [6, 7]. Several types of cytotoxic chemotherapy have been evaluated in patients with FLC, but most of the knowledge about the effectiveness of systemic therapy is based on anecdotal evidence and single-patient experience. Furthermore, factors that predispose individuals to FLC have not been defined and the number of affected individuals is very limited, resulting in a lack of standard care [8]. There are currently no specific recommendations or guidelines for the systemic treatment of high-risk FLC, which includes unresectable, relapsed, progressive, or metastatic disease [9, 10].

All FLCs harbor a deletion of ~400 kB in chromosome 19, resulting in a fusion of the genes for the heat shock protein DNAJ (Hsp40) homolog, subfamily B, member 1 (DNAJB1), and the catalytic subunit of protein kinase A (PRKACA) [11, 12]. Molecular analyses designed to identify putative therapeutic targets have also identified increased signaling through multiple pathways including, but not limited to, HER2 (*ERBB2*) [12, 13] and mammalian target of rapamycin (mTOR) [14]. HER2 kinase inhibitors have not been systematically evaluated in FLC to date. Early success with mTOR inhibition in a case report [15] did not translate into clinical benefits in a randomized phase 2 study among patients with advanced FLC [16]. Efforts to more directly target the DNAJB1-PRKACA chimera or its downstream effectors continue to evolve. Inhibition of aurora kinase A, which is overexpressed as a result of the *DNAJB1-PRKACA* gene fusion, had limited efficacy in a phase 2 study [17], whereas promising results were reported in a case study with a peptide-based immunotherapy targeting the DNAJB1-PRKACA fusion transcript [18].

Upregulation of multiple immune checkpoints has also been reported in FLC and provides the basis for investigating immune checkpoint blockade as a possible treatment option [19]. Clinical experience with immunotherapy in patients with FLC remains limited with conflicting results. No responses were documented with either single-agent immunotherapy (pembrolizumab or nivolumab) or a doublet combination (atezolizumab plus bevacizumab) in published case reports [20, 21]. However, promising findings were reported with a triplet combination of nivolumab, [peg]interferon alpha-2b, and fluoropyrimidine in patients with high-risk FLC in a recent retrospective study [22].

SUMMIT (Clinicaltrials.gov identifier NCT01953926) is an international, open-label, phase 2 "basket" study that investigated the efficacy and safety of neratinib, an irreversible pan-HER kinase inhibitor, across a broad spectrum of cancer lineages in patients whose tumors harbor activating *HER2* somatic mutations [23]. In the initial report from that study, clinical benefit rates ranged from 0% for ovarian cancer to 60% for patients with cervical cancer following treatment with neratinib monotherapy [23]. Furthermore, patients with breast cancer had a response rate of 24% and those with cervical cancer had a response rate of 20%, indicating the potential activity of neratinib monotherapy [23]. Although oncogenic *HER2* single nucleotide variants have not been reported in patients with FLC, given the rarity of this cancer type, an FLC cohort was added to SUMMIT in order to streamline evaluation of neratinib in this setting as FLC consistently exhibits upregulation of HER2 mRNA [11]. Patients were eligible for the FLC cohort irrespective of their *HER2* mutational status, as neither the extent nor the depth of HER2 expression was

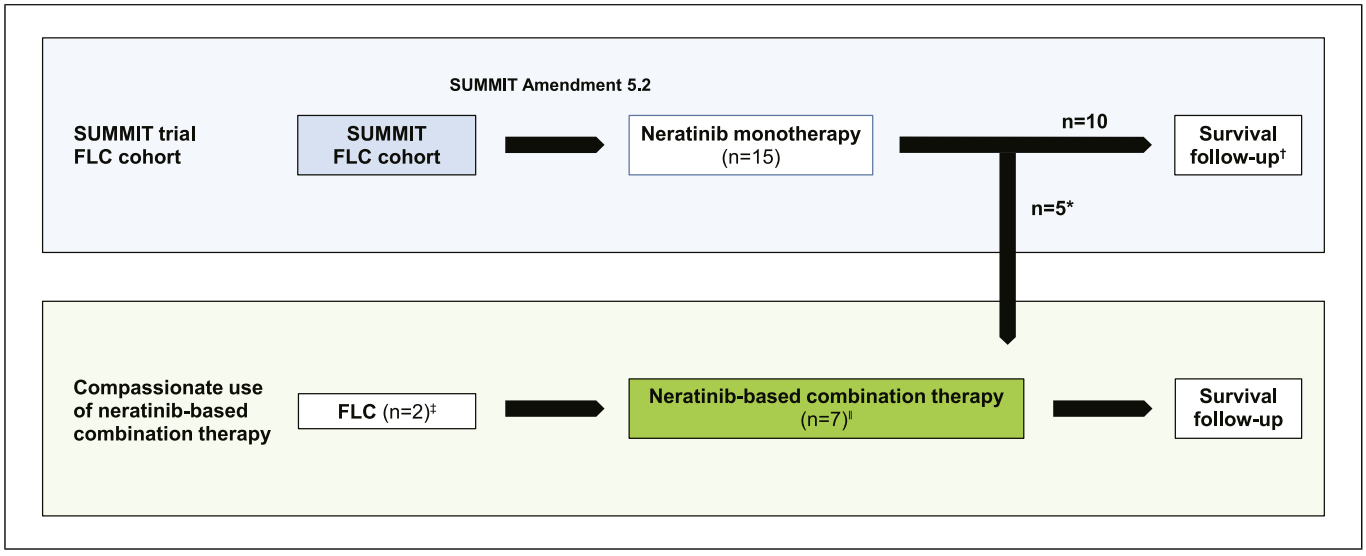

Fig. 1. Flowchart of patients with FLC in the SUMMIT study and Memorial Sloan Kettering Cancer Center compassionate-use program. *Five patients from SUMMIT switched to neratinib-based combination therapy under compassionate use following disease progression on neratinib monotherapy. †Patients were not followed for overall survival after January 4, 2022. ‡Two patients initiated neratinib-based combination therapy under compassionate use. ‖Neratinib in combination with immune checkpoint inhibitors (pembrolizumab or nivolumab) and/or mTOR inhibitors (everolimus or sunitinib). One patient each received pembrolizumab monotherapy and everolimus monotherapy during their treatments. FLC, fibrolamellar carcinoma; mTOR, mammalian target of rapamycin.

determined in this cohort and HER2 upregulation has been reported to be a potential distinguishing feature of FLC tumors [12]. Here, we report final results from this cohort of heavily pretreated patients with FLC who received single-agent neratinib as part of the SUMMIT study to determine whether HER2 inhibition has potential as a treatment strategy in FLC. We also report data from patients who were treated with neratinib in combination with an immune checkpoint inhibitor (pembrolizumab or nivolumab) and/or an mTOR inhibitor (everolimus or sunitinib) after progression on neratinib monotherapy or on a compassionate-use basis.

Methods

Study Design and Participants

This report describes patients with FLC who were enrolled in the SUMMIT study and received neratinib monotherapy or were treated with neratinib-based combination therapies as part of a compassionate-use program or upon progression after neratinib monotherapy (Fig. 1) [23]. Briefly, eligible patients were aged at least 18 years with cytologically confirmed FLC, and had disease that was evaluable using Response Evaluation Criteria in Solid Tumours (RECIST) version 1.1. Patients were required to have alanine transaminase and aspartate transaminase levels ≤5 × institutional upper limit of normal (ULN), total bilirubin ≤3 × ULN, creatinine ≤1.5 × ULN or creatinine clearance >60 cc/min, absolute neutrophil count ≥1,500 cells/mm^3, and platelets ≥75,000/mm^3.

Patients were eligible regardless of the number of prior lines of systemic therapy received. Compassionate-use patients were either treated with neratinib-based combinations as initial therapy or received neratinib-based combination therapy as part of a compassionate-use program following disease progression on neratinib monotherapy in the SUMMIT study (Fig. 1).

The SUMMIT study was conducted in accordance with the Declaration of Helsinki and approved by the Institutional Review Boards of participating institutions. Written informed consent was obtained from all patients before performing study-related procedures. The compassionate-use program was conducted as part of clinical care. Data for patients participating in the compassionate-use program were obtained retrospectively and anonymized as per Memorial Sloan Kettering Cancer Center Institutional Review Board approval.

Procedures

In the SUMMIT study, patients received neratinib 240 mg orally daily on a continuous basis and were treated until disease progression, unacceptable toxicity, or withdrawal of consent. In the compassionate-use program, patients received doublet or triplet combinations comprising neratinib 240 mg/day (with dose reductions depending on emergence of adverse events), pembrolizumab 2 mg/kg intravenously every 3 weeks, nivolumab 240 mg intravenously every 2 weeks, everolimus 7.5 mg orally once daily, or sunitinib 37.5 mg orally once daily. All patients received mandatory loperamide prophylaxis during cycle 1.

In the SUMMIT study, tumor response was assessed locally every 8 weeks by computed tomography, magnetic resonance imaging, and/or fluorodeoxyglucose-positron emission tomography. Patients with measurable disease according to RECIST version 1.1 were assessed primarily according to these criteria [24]. The same approach was taken for patients who were part of the compassionate-use program. Adverse events were classified according to Common Terminology Criteria for Adverse Events (version 4.0) [25] from consent until 28 days after discontinuation of study treatment.

Genomic Biomarker Studies

Patients were requested to submit either archival or pretreatment formalin-fixed, paraffin-embedded (FFPE) tumor tissue for biomarker studies. When available, tumor DNA was extracted from formalin-fixed, paraffin-embedded tissue or from plasma and sequenced using the Memorial Sloan Kettering-Integrated Mutation Profiling of Actionable Cancer Targets (MSK-IMPACT) assay [26]. Targeted RNA sequencing (Archer™; Boulder, CO, USA) was also performed in patients in whom the *DNAJB1-PRKACA* fusion was not detected through MSK-IMPACT because of low tumor content.

Outcomes

In SUMMIT, the primary endpoint was objective response rate (defined as either a complete or partial response confirmed no less than 4 weeks after response criteria were met). Secondary endpoints included clinical benefit rate (defined as confirmed complete response, partial response, or stable disease for at least 16 weeks), progression-free survival (defined as the interval from the start of treatment to the first date on which recurrence, progression, or death due to any cause was documented), and safety. Overall survival estimates for patients in SUMMIT are not provided, as they were no longer being followed for survival at the time of database lock. Patients in the compassionate-use program were followed for survival until death.

Statistical Analysis

In the SUMMIT study, a Simon two-stage optimal design was used to determine whether neratinib monotherapy had sufficient activity to warrant further development. In the first stage, enrollment continued until 7 patients had received at least one dose of study treatment and completed the first tumor assessment by the investigator (response evaluable). Additional enrollment beyond the first 7 patients was allowed to ensure that at least 7 patients were evaluable for radiographic response. If no responses were observed, the second stage for the cohort would be discontinued. Otherwise, 11 additional response-evaluable patients would be accrued for a total of 18 patients. A true ORR_{first} of ≤10% was considered unacceptable (null hypothesis) and a true ORR_{first} of minimally 30% (alternative hypothesis) with significance level 10% and power of 80% merited further study. The null hypothesis was rejected (for each cohort separately) if at least four responses were observed in the two stages. Baseline characteristics, efficacy, and safety data were summarized in the safety analysis set, which included all patients who received at least one dose of neratinib. The Clopper-Pearson method was used to calculate 95% confidence intervals (CIs) for response rates. Kaplan-Meier methodology was used to evaluate time-to-event endpoints. All statistical analyses were performed using SAS (version 9.4; SAS Institute Inc., Cary, NC, USA).

In the compassionate-use program, baseline characteristics and safety data were summarized in patients who received at least one dose of neratinib-based combination therapy. Best overall responses were presented for each patient according to the regimen received.

Results

Between July 19, 2018, and October 21, 2019, 15 patients with FLC were included in the SUMMIT study and received at least one dose of neratinib monotherapy. Additional enrollment beyond the first 7 patients, which was allowed to ensure that at least 7 patients were evaluable for radiographic response, resulted in over-enrollment in the study. At the time of data cut-off (January 4, 2023), all 15 patients had discontinued neratinib treatment (disease progression, $n = 9$; withdrawal of consent, $n = 3$; adverse events, $n = 1$; noncompliance, $n = 1$; clinical progression, $n = 1$). Five patients (33.3%) who developed progressive disease in the SUMMIT study went on to receive neratinib-based combination therapy under the compassionate-use program (Fig. 1). Two additional patients with advanced FLC initiated neratinib-based combination therapy under compassionate use (Fig. 1). Therefore, a total of 7 patients in the compassionate-use program received at least one dose of neratinib-based combination therapy. At the time of data cut-off, all 7 patients had discontinued combination therapy. Five patients (71.4%) died after disease progression.

Patient characteristics are summarized in Table 1. Among patients in the SUMMIT FLC cohort, the median age was 26 years, with a median time from diagnosis to study enrollment of 2.5 years. Twelve patients (80.0%) had prior surgery, and 9 (60.0%) had previously received an anticancer medication. Four patients (26.7%) had normal hepatic function, 10 (66.7%) had mild hepatic impairment, and 1 (6.7%) had moderate hepatic impairment [27]. Among patients treated with neratinib combination therapy on a compassionate-use basis, the median age at baseline was 26 years, with a median time from diagnosis to study enrollment of 3.4 years. Five of 7 patients (71.4%) had prior surgery, and 3 (42.9%) had previously received an anti-cancer medication (median number of lines, 1; range 1–5). The *DNAJB1-PRKACA* fusion was detected in all 4 patients with tissue available for next-generation sequencing (Table 1).

Among patients treated with neratinib monotherapy in the SUMMIT study ($n = 15$), the median time on treatment was 2.1 (interquartile range 0.9–4.1) months. There were no complete or partial responses (Table 2). Two patients (13.3%) had stable disease lasting at least 16 weeks, corresponding to a clinical benefit rate of 13.3% (95% CI: 1.7–40.5). Median progression-free survival was 3.6 months (95% CI: 1.8–3.7).

Among the 5 patients treated with neratinib-based combinations after neratinib monotherapy in SUMMIT,

Table 1. Baseline demographics and clinical characteristics of patients with FLC

Characteristic	SUMMIT	Compassionate-use program
	neratinib monotherapy (n = 15)	neratinib-based combination therapy[a] (n = 7)[b]
Median age (range), years	26 (19–50)	26 (17–40)
Sex, *n* (%)		
Male	9 (60.0)	4 (57.1)
Female	6 (40.0)	3 (42.9)
ECOG performance status, *n* (%)		
0	4 (26.7)	0
1	11 (73.3)	7 (100.0)
Median time from initial diagnosis to enrollment (range), years	2.5 (0.1–10.6)	3.4 (0.03–6.9)
Median time from first metastasis to enrollment (range), years	1.4 (0.0–2.9)	3.4 (0.03–6.9)
Prior therapy, *n* (%)		
Radiotherapy	3 (20.0)	0
Surgery	12 (80.0)	5 (71.4)
Anticancer medication	9 (60.0)	3 (42.9)
Cytotoxic therapy	7 (46.7)	2 (28.6)
Targeted therapy	4 (26.7)	1 (14.3)
Immunotherapy	1 (6.7)	1 (14.3)
Median No. prior systemic therapies (range)	2 (1–5)	0 (0–4)
Genetic alterations, *n* (%)	(n = 4)	(n = 4)
DNAJB1-PRKACA fusion (among tested)	4 (100.0)	4 (100.0)

ECOG, Eastern Cooperative Oncology Group. [a]Neratinib plus pembrolizumab, nivolumab or everolimus, or neratinib plus pembrolizumab plus everolimus. [b]Includes 2 patients who were not initiated on neratinib monotherapy (outside SUMMIT) and 5 patients who switched to neratinib-based combination therapy following progression of their disease on neratinib monotherapy in the SUMMIT study.

three received neratinib plus pembrolizumab after progression, one received neratinib plus nivolumab, and one received neratinib plus everolimus. Best overall responses for the 7 patients treated with neratinib-based combination therapy are presented in online supplementary Table 1 (for all online suppl. material, see https://doi.org/10.1159/000540290). Durations of response to combination treatment are shown in Figure 2. Four of 5 patients who had combination therapy received treatment for over 1 year, with addition and withdrawal of agents according to observed responses. One patient (SUMMIT #2; 1726) who received neratinib plus pembrolizumab after neratinib monotherapy in SUMMIT had a best response of partial response lasting 3 months. Addition of everolimus to neratinib plus pembrolizumab resulted in a further stable disease lasting 6 months. One patient (SUMMIT #1) who progressed on neratinib monotherapy and subsequently received neratinib plus pembrolizumab had stable disease lasting 3 months. The patient later received neratinib plus everolimus followed by neratinib plus pembrolizumab plus everolimus and had a second stable disease of 9 months' duration. A third patient (SUMMIT #4) who progressed on neratinib monotherapy subsequently received neratinib plus everolimus and had stable disease lasting 5 months. One of 2 patients who did not participate in SUMMIT (COM1) received neratinib plus pembrolizumab, followed by neratinib plus pembrolizumab plus everolimus, but experienced an allergic reaction to everolimus and switched to neratinib plus pembrolizumab plus sunitinib. This patient remained on treatment for 26 months overall and had a best response of stable disease.

Details of the patient with a partial response during neratinib plus pembrolizumab treatment are shown in online supplementary Figure 1. Among 15 patients who received neratinib monotherapy in the SUMMIT study, 14 (93.3%) had at least one treatment-emergent adverse event (online suppl. Table 2). Ten patients (66.7%) had a

Table 2. Efficacy of neratinib monotherapy in the SUMMIT study (n = 15; efficacy-evaluable population per RECIST)

	Neratinib monotherapy (n = 15)
Objective response at week 8, n[a]	0
CR	0
PR	0
Objective response rate, % (95% CI)	0 (0.0–21.8)
Overall objective response (confirmed CR or PR), n[b]	0
CR	0
PR	0
Objective response rate, % (95% CI)	0 (0.0–21.8)
Duration of response (95% CI), months	NA
Best overall response (confirmed and unconfirmed CR or PR), n[b]	0
CR	0
PR	0
Best overall response rate, % (95% CI)	0 (0.0–21.8)
Clinical benefit rate, n (%)[c]	2 (13.3)
CR	0
PR	0
SD ≥16 weeks, n (%)	2 (13.3)
Clinical benefit rate, % (95% CI)	13.3 (1.7–40.5)
Progression-free survival	
Median (95% CI), months	3.6 (1.8–3.7)

CI, confidence interval; CR, complete response; NA, not applicable; PR, partial response; RECIST, Response Evaluation Criteria in Solid Tumours; SD, stable disease. [a]Objective response at week 8 is defined as CR or PR at 8 weeks of study therapy, which corresponds to the first scheduled tumor assessment. [b]Objective response is defined as either a CR or PR that is confirmed no less than 4 weeks after the criteria for response are initially met. [c]Clinical benefit rate is defined as confirmed CR or PR or SD for ≥16 weeks (within ±7-day visit window).

grade 3 adverse event and 2 (13.3%) had a grade 4 event. Diarrhea was the most common adverse event: 11 patients (73.3%) reported diarrhea of any grade and 4 (26.7%) had a grade 3 diarrhea event; no grade 4 diarrhea was reported. Other common adverse events included fatigue (n = 8; 53.3%) and nausea (n = 7; 46.7%).

Treatment-related adverse events with neratinib-based combination therapy are summarized in online supplementary Table 3. Among 5 patients who received neratinib in combination with pembrolizumab, common treatment-related grade 1 or 2 adverse events were diarrhea and fatigue (4 patients each), and 1 patient (16.7%) had a grade 3 adverse event (hyperglycemia). In 2 patients who received neratinib in combination with everolimus, only grade 1 adverse events were observed, which included diarrhea, fatigue, abdominal pain, maculopapular rash, and mucositis. Grade 1 fatigue was reported in all 3 patients treated with the triplet combination of neratinib plus pembrolizumab plus everolimus. Other adverse events reported in 1 patient each with this triplet were maculopapular rash (grade 1), mucositis (grade 2), anaphylaxis (grade 3), and hepatic failure (grade 3). One patient discontinued everolimus after 2 weeks because of grade 3 anaphylaxis and received neratinib plus pembrolizumab plus sunitinib, which was well tolerated with grade 1 adverse events only (abdominal pain, fatigue, maculopapular rash, and hair discoloration). No grade 4 or 5 adverse events were reported with any combination regimen.

Discussion

This report describes outcomes in patients with advanced FLC who received pan-HER kinase inhibition with neratinib as monotherapy in the SUMMIT study and pan-HER kinase inhibition as part of a combination regimen

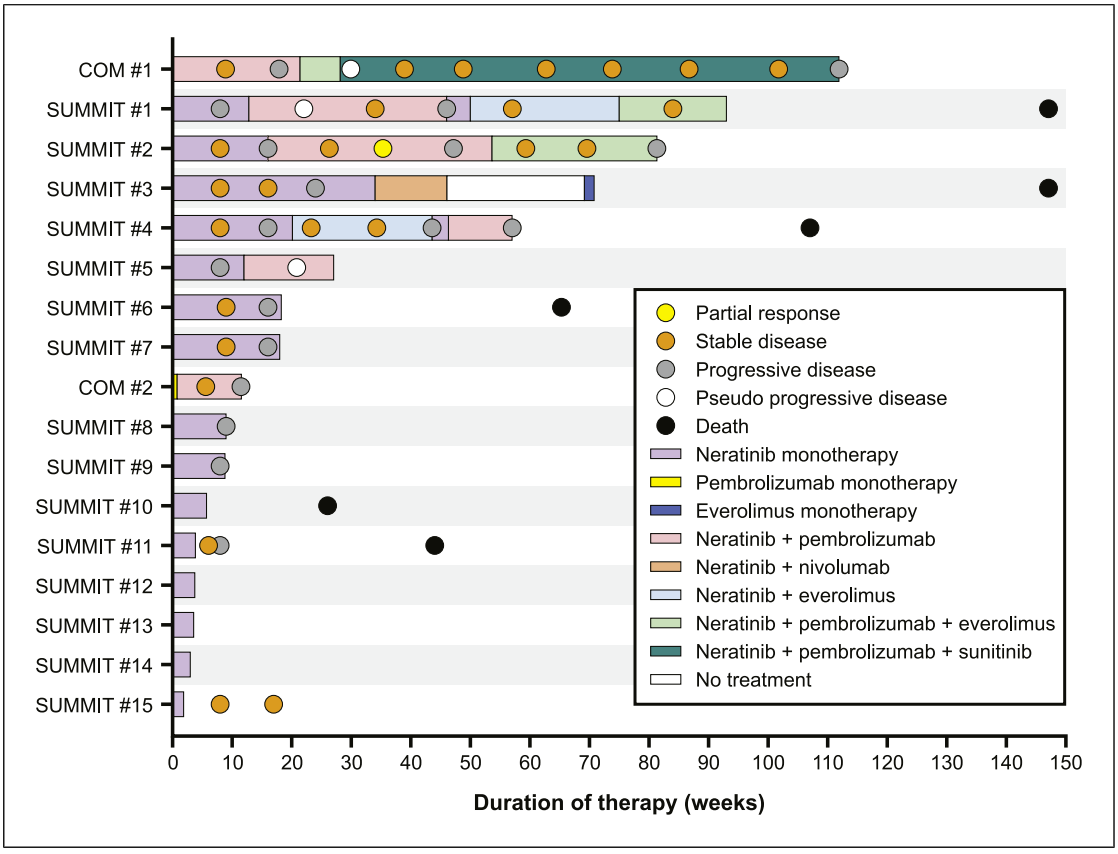

Fig. 2. Duration of therapy in patients with FLC who received neratinib monotherapy (SUMMIT) or neratinib-based combination therapy (compassionate use).

with an immune checkpoint inhibitor and/or an mTOR inhibitor in a compassionate-use program. Although transcriptomic activation of HER2-linked pathways has been reported to be increased in FLC [12], single-agent neratinib had limited clinical benefit (clinical benefit rate, 13.3% in this setting). However, neither HER2 mRNA nor protein expression were measured in this cohort of patients, and neither HER2 gene copy-number alterations nor mutations were detected in any of the tumors from samples with next-generation sequencing reports. Evidence of clinical benefit was observed upon addition of the immune checkpoint inhibitor pembrolizumab to neratinib. A partial response was observed in 1 patient treated with this combination and 3 patients had stable disease lasting 3–9 months. Addition of everolimus or sunitinib to neratinib plus pembrolizumab further extended the duration of stable disease in 3 patients. The treatment regimens examined in this study were diverse and the contribution of any one agent to the efficacy in any given patient remains unclear. Furthermore, the patients and their disease histories were also varied, the latter encompassing a range of prior therapies; these factors may also have played a role in determining the outcome of treatments administered in this study. Nonetheless, it cannot be excluded that the responses observed were due to the immune checkpoint or mTOR inhibition alone [28], and these cases suggest that a combination of pan HER-targeted therapy plus immunotherapy and/or mTOR-targeting agents merits further study given the continued lack of an effective standard-of-care systemic therapy for patients with unresectable or recurrent FLC.

Some cases of FLC have been reported to display multiple immune-suppressive mechanisms, including upregulation of programmed death-ligand 1 expression on tumor cell membranes and programmed death-1 expression on tumor-infiltrating lymphocytes and tumor-associated macrophages [19]. As immune evasion appears to be a feature of FLC oncogenesis, immunotherapy is a rational treatment strategy for clinical testing. However, no treatment successes were reported in previous studies in patients with FLC who were treated with either immune checkpoint inhibitor monotherapy

[20, 29] or with atezolizumab plus bevacizumab [21], a regimen with demonstrated efficacy in hepatocellular carcinoma. A possible explanation for the lack of efficacy in prior studies may be that the patients treated with these regimens were poor candidates for therapy. Alternatively, combination regimens targeting different immune checkpoint proteins may be required to trigger an effective immune response in patients with FLC. Promising results (objective response rate, 50%; tumor control rate, 93%) have been described for a triplet combination of nivolumab, (peg)interferon alpha-2b, and a fluoropyrimidine in patients with high-risk FLC in a retrospective study [23]. A prospective phase 1/2 study of the nivolumab, fluorouracil, and interferon alpha-2b combination for unresectable FLC is underway (NCT04380545). These findings, together with our own, suggest that combination treatments involving immunotherapy hold promise for the treatment of patients with this cancer.

The question of whether outcomes are improved by adding an mTOR inhibitor to immunotherapy or HER2 inhibition remains unanswered. It is encouraging that the addition of everolimus to neratinib and pembrolizumab extended the duration of stable disease in 1 patient. A controlled study would be required to provide further insight into the effectiveness of combination regimens involving mTOR inhibitors in patients with FLC. Another approach worthy of consideration is simultaneous targeting of the presumed primary genetic driver for FLC, the *DNAJB1-PRKACA* chimera, and critical downstream pathways. Future directions for FLC research are likely to focus on combinatorial approaches that include molecular therapies and immunotherapy, and definition of the patient population to derive the best outcomes from any clinical trials [30].

The neratinib-based combination regimens investigated were considered tolerable, with no treatment-related grade 4 or 5 events reported. Most adverse events were mild to moderate in severity, and grade 3 events were isolated. The safety profile of neratinib in combination with pembrolizumab was characterized by grade 1/2 diarrhea and fatigue, with one case of grade 3 hyperglycemia, all adverse events observed previously with these agents. These safety data support a starting dose of neratinib 240 mg/day with dose reductions if needed, combined with the pediatric dose of pembrolizumab (2 mg/kg) given every 3 weeks.

This exploratory dataset has several limitations. DNAJB1-PRKACA fusion protein is a hallmark of FLC; however, tissue genomic sequencing was performed in only 4 patients, in whom this fusion was detected. Further, neither HER2 expression levels nor signaling pathways were assessed in any of the patient samples; therefore, HER2 pathway upregulation could not be confirmed in the patients treated. In patients who received neratinib plus immunotherapy, clinical benefit to immunotherapy on its own, rather than the combination per se, cannot be excluded. Other limitations include small patient numbers and the retrospective analysis of data from patients included in the compassionate-use program. The rarity of FLC poses an ongoing challenge to conducting clinical research and the accrual of sufficient patient numbers.

In conclusion, monotherapy with a HER2-directed tyrosine kinase inhibitor had limited efficacy in patients with FLC. Clinical benefit was observed in patients receiving neratinib plus pembrolizumab with or without everolimus. These are case-limited observations but may be worth evaluating further in upcoming clinical trials given the continued lack of therapeutic options for patients with FLC.

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Statement of Ethics

The study was approved by Ethics Committees at the participating institutions: Memorial Sloan Kettering Cancer Center IRB/Privacy Board – A, IRB 13-140; The University of Texas MD Anderson Cancer Center IRB #5 – Clinical; UCSF Human Research Protection Program IRB; NHS Health Research Authority, London-Hamstead Research Ethics Committee, No. 15/LO/0844. All patients provided written informed consent to participate in the study.

Conflict of Interest Statement

Ghassan K. Abou-Alfa reports institutional research support from Arcus, Agios, AstraZeneca, BioNTech, BMS, Celgene, Flatiron, Genentech/Roche, Genoscience, Incyte, Polaris, Puma, QED, Silenseed, and Yiviva, consulting support from Adicet, Alnylam, AstraZeneca, Autem, Bayer, Beigene, Berry Genomics, Celgene, Cend, CytomX, Eisai, Eli Lilly, Exelixis, Flatiron, Genentech/Roche, Genoscience, Helio, Incyte, Ipsen, Legend Biotech, Merck, Nerviano, QED, Redhill, Rafael, Servier, Silenseed, Sobi, Surface Oncology, Therabionics, Vector, and Yiviva, and a patent (International Patent Application No. PCT/US2014/031545 filed on March 24, 2014, and priority application Serial No. 61/804,907 filed March 25, 2013). Tim Meyer reports receiving grants from MSD and consulting fees (personal) from Eisai, BMS, Adaptimmune, Ipsen, Roche, AstraZeneca, MSD, and Beigene. Richard Kinh Gian Do, Joseph Light, Amin Yaqubie, Alison

Clemens O'Neill, Pablo Cano, and Albert S. Cornelius have no conflicts to declare. Sarina A. Piha-Paul reports receiving clinical trial research support/grant funding through the institution from AbbVie, Inc., ABM Therapeutics, Inc., Acepodia, Inc, Alkermes, Aminex Therapeutics, Amphivena Therapeutics, Inc., BioMarin Pharmaceutical, Inc, Boehringer Ingelheim, Bristol Myers Squib, Cerulean Pharma, Inc., Chugai Pharmaceutical Co., Ltd, Curis, Inc., Cyclacel Pharmaceuticals, Daiichi Sankyo, Eli Lilly, ENB Therapeutics, Epigenetix Inc., Five Prime Therapeutics, F-Star Beta Limited, F-Star Therapeutics, Gene Quantum, Genmab A/S, Gilead Sciences, Inc., GlaxoSmithKline, Helix BioPharma Corp., Hengrui Pharmaceuticals, Co., Ltd., HiberCell, Inc., Immorna Biotherapeutics, Inc., Immunomedics, Inc., Incyte Corp., Jacobio Pharmaceuticals Co., Ltd., Jiangsu Simcere Pharmaceutical Co., Ltd., Lytix Biopharma AS, Medimmune, LLC., Medivation, Inc., Merck Sharp and Dohme Corp., Nectin Therapeutics, Ltd., Novartis Pharmaceuticals, Pieris Pharmaceuticals, Inc., Pfizer, Phanes Therapeutics, Principia Biopharma, Inc., Puma Biotechnology, Inc., Purinomia Biotech, Inc., Rapt Therapeutics, Inc., Replimune, Seattle Genetics, Silverback Therapeutics, Synlogic Therapeutics, Taiho Oncology, Tesaro, Inc., TransThera Bio, ZielBio, Inc., NCI/NIH, P30CA016672 – Core Grant (CCSG Shared Resources), and consulting fees from CRC Oncology. Scott Sherrin reports stock or stock options in Pfizer, Inc. James J. Harding reports receiving institutional research support from Bristol Myers Squibb, Boehringer Ingelheim, CytomX, Calithera, Eli Lilly, Genoscience, Incyte, Loxo, Novartis, Pfizer, Yiviva, and Zymeworks and has consulted for Adaptimmune, Bristol Myers Squibb, CytomX, Eisai, Eli Lilly, Exelixis, Merck, QED, and Zymeworks. Raed Al Rajabi reports receiving grants or contracts (to institution) from AstraZeneca, Bayer, Exalixis, BioMed Valley Discoveries, and Eureka Therapeutics, participating in a data safety monitoring board or advisory board for AstraZeneca and Taiho, and holding stock or stock options in Seagen and Actinium Pharmaceuticals. Crystal S. Denlinger reports grants or contracts (to institution) from Amgen, AstraZeneca, Agios, Bristol Meyers Squibb, Beigene, Exelixis, Genmab, Zymeworks, Sanofi, and MedImmune, participating in a data safety monitoring board or advisory board for Zymeworks, Beigene, Bristol Meyers Squibb, Merck, and Taiho Oncology, and receipt of medical writing support from Beigene and Amgen. Eileen M. O'Reilly reports receiving research funding to institution from Genentech/Roche, BioNTech, AstraZeneca, Arcus, Elicio, Parker Institute, NIH/NCI, and Pertzye and fees for consulting/data safety monitoring board from Boehringer Ingelheim, BioNTech, Ipsen, Merck, Novartis, AstraZeneca, BioSapien, Astellas, Thetis, Autem, Novocure, Neogene, BMS, Tempus, Fibrogen, Merus, Agios (to spouse), Genentech-Roche (to spouse), and Eisai (to spouse). Daniel DiPrimeo and Lisa D. Eli are employees of and stockholders in Puma Biotechnology, Inc. John D. Gordan reports receiving grants or contracts from the Fibrolamellar Cancer Foundation, support for attending meetings and/or travel from the Fibrolamellar Cancer Foundation and the Cholangiocarcinoma Foundation, patent WO/2021/155004, a leadership or fiduciary role in the Fibrolamellar Cancer Foundation and ASCO; receipt of equipment, drugs, materials, medical writing, gifts or other services from Mirati and eFFECTOR. David B. Solit has consulted/received honoraria from Rain, Pfizer, Fog Pharma, PaigeAI, BridgeBio, Scorpion Therapeutics, FORE Therapeutics, Function Oncology, Pyramid, and Elsie Biotechnologies, Inc.

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Author Contributions

Ghassan K. Abou-Alfa and John D. Gordan contributed to the conception or design of the work. Ghassan K. Abou-Alfa, Richard Kinh Gian Do, Scott Sherrin, Amin Yaqubie, Raed Al Rajabi, and Lisa D. Eli contributed to the acquisition, analysis, or interpretation of data for the work. Ghassan K. Abou-Alfa, Tim Meyer, Richard Kinh Gian Do, Sarina A. Piha-Paul, Joseph S. Light, Scott Sherrin, Amin Yaqubie, Alison Clemens O'Neill, James J. Harding, Raed Al-Rajabi, Crystal S. Denlinger, Pablo Cano, Albert S. Cornelius, Eileen M. O'Reilly, Daniel DiPrimeo, Lisa D. Eli, John D. Gordan, and David B. Solit were involved in interpretation of data for the work, drafting or revising the report critically for important intellectual content, and final approval of the version to be published. Ghassan K. Abou-Alfa, Tim Meyer, Richard Kinh Gian Do, Sarina A. Piha-Paul, Joseph S. Light, Scott Sherrin, Amin Yaqubie, Alison Clemens O'Neill, James J. Harding, Raed Al-Rajabi, Crystal S. Denlinger, Pablo Cano, Albert S. Cornelius, Eileen M. O'Reilly, Daniel DiPrimeo, Lisa D. Eli, John D. Gordan, and David B. Solit agreed to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. Ghassan K. Abou-Alfa, Tim Meyer, Richard Kinh Gian Do, Sarina A. Piha-Paul, Joseph S. Light, Scott Sherrin, Amin Yaqubie, Alison Clemens O'Neill, James J. Harding, Raed Al-Rajabi, Crystal S. Denlinger, Pablo Cano, Albert S. Cornelius, Eileen M. O'Reilly, Daniel DiPrimeo, Lisa D. Eli, John D. Gordan, and David B. Solit had full access to all the data in the study and accept responsibility to submit the publication. Ghassan K. Abou-Alfa, Joseph Light, Scott Sherrin, Lisa D. Eli, and Amin Yaqubie directly accessed and verified the underlying data reported in the manuscript.

Data Availability Statement

The authors declare that the data supporting the findings of this study are available within the article. Qualified researchers and study participants may submit requests for other study documentation and clinical trial data to clinicaltrials@pumabiotechnology.com for consideration.

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