Cancer Treatment and Research Communications 21 (2019) 100158

Contents lists available at ScienceDirect

Cancer Treatment and Research Communications

journal homepage: www.elsevier.com/locate/ctarc

Phase Ib/II study of hydroxychloroquine in combination with chemotherapy in patients with metastatic non-small cell lung cancer (NSCLC)

Jyoti Malhotra*, Salma Jabbour, Michelle Orlick, Gregory Riedlinger, Yanxiang Guo, Eileen White, Joseph Aisner

Medical Oncology, Rutgers Cancer Institute of New Jersey, Robert Wood Johnson Medical School, 195, Little Albany street, New Brunswick, NJ 08903, USA

ARTICLE INFO

Keywords:
Lung cancer
Autophagy
Hydroxychloroquine

ABSTRACT

Objectives: Activation of cell survival pathways such as autophagy represents a potential resistance mechanism to chemotherapy in NSCLC. Preclinical studies report that autophagy inhibition suppresses lung tumor development and progression. We report the safety and efficacy for adding autophagy inhibitor, hydroxychloroquine, to chemotherapy in a phase Ib/II single-arm study in patients with metastatic NSCLC.

Patients and methods: We treated patients with untreated metastatic NSCLC with carboplatin, paclitaxel (and bevacizumab if criteria met) and hydroxychloroquine 200 mg BID. Patients continued on hydroxychloroquine (+/− bevacizumab) maintenance after 4–6 cycles of therapy.

Results: We enrolled 40 patients, 8 on phase Ib and 32 on phase II. Forty-three percent were female; 50% with squamous histology. Median age was 62 years (range, 43–73). Thirteen patients developed ≥grade 3 treatment-related adverse event. Common adverse events (all grades) were neutropenia (35%), neuropathy (32.5%), and anemia (32.5%). The objective response rate (ORR) was 33% in the 30 patients (phase II) evaluable for response. Additionally, 20% of the patients demonstrated stable disease (clinical benefit rate of 53%). The median PFS was 3.3 months (95% CI 2.1–6.8 months). In 9 patients with *KRAS* positive tumors, ORR was 44% and median PFS was higher than expected at 6.4 months (95% CI 1.8–15.6).

Conclusions: Addition of hydroxychloroquine is safe and tolerable with a modest improvement in clinical responses compared to prior studies. Autophagy inhibition may overcome chemotherapy resistance in advanced NSCLC and further study in a more molecularly selected population such as *KRAS*-positive tumors is warranted.

Introduction

Lung cancer is the leading cause of cancer-related mortality in the United States, with an estimated 155,870 deaths in 2017 [1]. Over 85% of these patients will have had non-small cell lung cancer (NSCLC), and the majority of these patients presented with advanced disease at the time of diagnosis. Those patients with advanced disease treated with platinum-based doublet historically show a median survival of approximately 8 months and a 1-year survival rate of only 33–38% [2,3]. The lack of durable responses with platinum-based chemotherapy points towards resistance mechanisms. Activation of cell survival pathways such as autophagy represents one such possible resistance mechanism. Autophagy is a normal, well-regulated cellular response that activates during times of nutrient deprivation. Autophagy is a recycling program, whereby autophagosomes containing sequestered cytoplasmic material fuse with lysosomes, resulting in digestion and reutilization of these cellular contents [4]. This process temporarily maintains cellular energy, limits the accumulation of damaged proteins and organelles during cellular stress, and thereby promotes cellular survival. Tumors and tumor cells, particularly those driven by oncogenic *KRAS* and *BRAF*, upregulate autophagy even in the fed state and depend on if for survival and maligancy [5–8]. Thus, blocking autophagy represents a potential means to increase tumor cell death in cancers driven by different oncogenic events. Preclinical studies [9–12] in engineered mouse lung cancer models also show that *KRAS* mutant lung tumors depend on autophagy, and inhibition of autophagy inhibits tumor development and progression.

Hydroxychloroquine is a 4-aminoquinoline in use for more than 30 years for the treatment of malaria, rheumatoid arthritis, and systemic lupus erythematosus. This agent works through inhibition of lysosomal acidification, which blocks their terminal step of autophagic substrate degradation. The addition of chloroquine or hydroxychloroquine to either antiangiogenic agents or cytotoxic agents may significantly increase antitumor activity. Multiple trials combined anticancer therapies

* Corresponding author.
E-mail address: jyoti.malhotra@rutgers.edu (J. Malhotra).

https://doi.org/10.1016/j.ctarc.2019.100158

such as chemotherapy and radiation therapy with hydroxychloroquine, and reported that autophagy inhibition appears achievable with hydroxychloroquine [13–16]. Therefore, we developed an open-label Phase Ib/II study of paclitaxel, carboplatin, and bevacizumab (in Bevacizumab eligible patients) plus hydroxychloroquine for the treatment of patients with advanced NSCLC to test our hypothesis that inhibition of autophagy using hydroxychloroquine will improve the activity of standard chemotherapy (with or without bevacizumab) in this patient population. Our Phase I study (NCT00728845) already showed tolerability at standard doses of all agents.

Patients and methods

Eligibility criteria

Subjects needed histologically or cytologically confirmed advanced stage NSCLC (stage IV). Other key inclusion criteria included: age 18 years or older, measurable disease by Response Evaluation Criteria in Solid Tumors (RECIST) 1.1 criteria, no prior therapy in advanced or metastatic setting, and adequate hematologic, hepatic, and renal function. Patients with adequately treated and stable brain metastases were eligible. Exclusion criteria included: known HIV infection; medical, social, or psychological factors affecting safety or compliance; grade 2 or higher neuropathy; history of hypersensitivity to any of the study drugs or 4-aminoquinoline compounds; pregnant/breastfeeding; active infection and or known or suspected G-6P deficiency. Additionally, eligibility for Cohort 1 (bevacizumab eligible) required non-squamous histology, no history of hemoptysis within 3 months, no significant cardiovascular event within 6 months, no history of abdominal fistula or perforation within 6 months, and no history of uncontrolled hypertension. We enrolled the patients at Rutgers Cancer Institute of New Jersey, Robert Wood Johnson University Hospital at New Brunswick and at Hamilton. We also required a signed written informed consent form, approved by our Institutional Review Board. The trial was registered at clinicaltrials.gov: NCT00728845 and NCT01649947.

Treatment and efficacy assessments

This is an open-label Phase Ib/II study of the combination of paclitaxel at 200 mg/m^2 intravenously over 3 h on Day 1; followed by carboplatin AUC = 6 intravenously over 15–30 min on Day 1 followed by Bevacizumab (for Cohort 1) at 15 mg/kg intravenously (initial infusion over 90 min) on Day 1; and hydroxychloroquine orally (200 mg BID) on Days 1–21. Chemotherapy cycles were planned every 21 days for a total of 6 cycles if tolerated. Patients in Cohort 1 then continued to receive bevacizumab every 21 days and hydroxychloroquine daily up to 1 year until we found evidence of disease progression or unacceptable toxicity. Patients in Cohort 2 received hydroxychloroquine alone (without bevacizumab) for up to 1 year. After baseline evaluation, we evaluated tumor response after every 2 cycles (6 weeks) of therapy using contrast enhanced CT scan. We assessed response using RECIST 1.1 criteria. We followed survival every 2–3 months after completion of initial chemotherapy until progression of disease, and then followed for overall survival. All patients who received one dose of protocol therapy were evaluable for assessment of toxicity. All patients who received one cycle of chemotherapy and at least 75% of the oral hydroxychloroquine during cycle 1 were evaluable for efficacy. Safety assessments included symptoms, vital signs, laboratory assessments, and physical examinations. AEs were assessed using the National Cancer Institute Common Terminology Criteria for Adverse Events (CTCAE v3.0). We allowed up to two dose reductions for paclitaxel (175 and 150 mg/m^2) and carboplatin (AUC = 5 and AUC = 4). Subjects requiring additional dose reductions were removed from study.

Biomarker measurements

We evaluated optional archival tumor specimens for molecular alterations including *KRAS, EGFR, ALK* and *ROS-1*.

Statistical analysis

The primary objective of this study was to assess the antitumor activity, as measured by tumor response frequency, of paclitaxel, carboplatin, Bevacizumab (for eligible patients) and hydroxychloroquine in patients with advanced or recurrent NSCLC. The secondary endpoints of this study included progression-free survival (PFS), incidence and severity of adverse events, and an exploratory end-point of *KRAS* status) correlation with efficacy in patients receiving this regimen. We tested the hypothesis that the addition of hydroxychloroquine to the paclitaxel, carboplatin and bevacizumab combination could increase the response rate from 30% to 60%. We used a Simon's two-stage minimax design with a 5% significance level and 80% power. This required a minimum of 8 patients during the first stage and 12 patients in the second; with a maximum sample size were 20. If we observed 2 or fewer responses during phase 1b, then the trial was to be stopped early for futility. If we found 3 or more responses, the trial was to continue to phase II. Therefore, at least 20 patients evaluable for efficacy assessment were targeted to enroll in at least one of the cohorts. For the assessment of PFS, we used Kaplan–Meier estimates of survival function and calculated the standard errors. The data cut-off for analysis was December 31st 2016.

Results

Baseline characteristics

We enrolled 8 patients on phase Ib followed by 32 patients on phase II part between February 2009 and June 2015. Of these, we treated 20 patients as per the Cohort 1 treatment plan (received bevacizumab; 8 on phase Ib and 12 on phase II) as they had non-squamous histology, no history of hemoptysis within 3 months, no significant cardiovascular event within 6 months, no history of abdominal fistula or perforation within 6 months, and no history of uncontrolled hypertension. Additionally, 20 patients who did not meet these criteria for bevacizumab were treated as per the Cohort 2 treatment plan (did not receive bevacizumab; all on phase II) (Table 1). The median age of the patients was 62 years (range 43–73 years) and 43% were female. Fifty percent of the patients had squamous cell carcinoma. All patients with

Table 1
Patient demographics.

	All (n = 40)	Study phase		Treatment arm	
		Phase Ib (n = 8)	Phase II (n = 32)	Cohort 1 (n = 20)	Cohort 2 (n = 20)
Median age (range)	62 (43–74)	65 (48–74)	62 (43–73)	62 (43–74)	62 (51–73)
Gender					
Female	17	3	14	9	8
Male	23	5	18	11	12
Race/ethnicity					
Caucasian	34	6	28	16	18
African American	6	2	4	4	2
Histology					
Non-squamous	20	8	18	20	0
Squamous	20	0	14	0	20

Cohort 1: treatment with carboplatin, paclitaxel, hydroxychloroquine and bevacizumab.
Cohort 2: treatment with carboplatin, paclitaxel, hydroxychloroquine only.

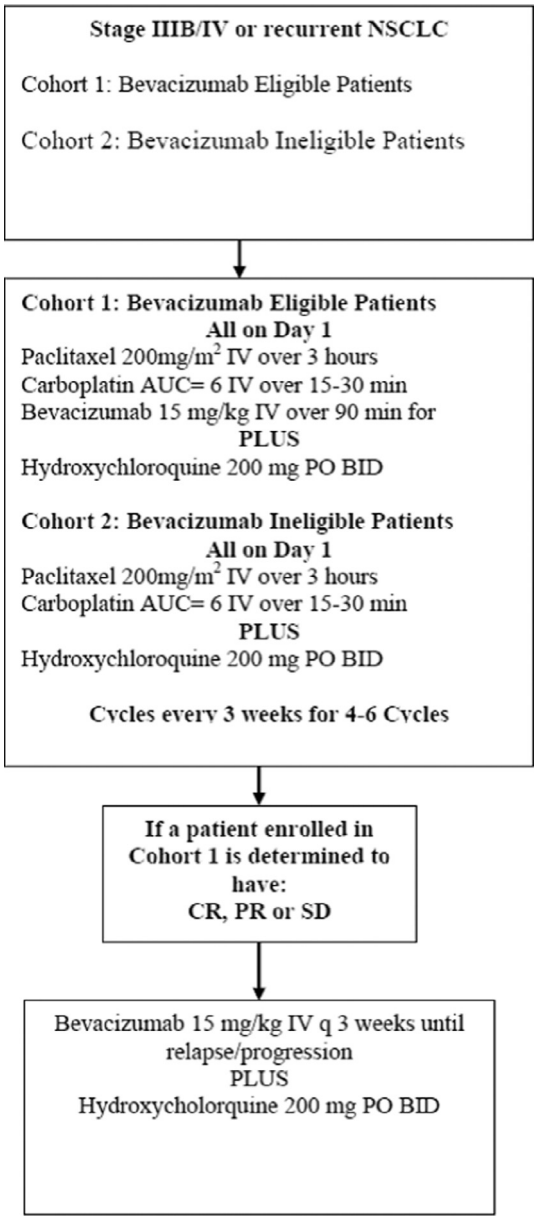

Fig. 1. Study schema.

squamous cell type were on Cohort 2 treatment arm and did not receive bevacizumab. The study schema is explained in Fig. 1.

Efficacy evaluation

Thirty of the 32 patients enrolled on the phase II were evaluable for efficacy (12 in Cohort 1 and 18 in Cohort 2). No patient had complete response with therapy. Two patients were not evaluable as they came off trial therapy during cycle 1 for non-compliance and for non-treatment related comorbidity. ORR (complete response plus partial response) was 33% overall (Table 2). We found no difference in ORR by histology (35% in non-squamous and 31% in squamous cell carcinoma). However, patients treated with bevacizumab showed a higher ORR compared to those who did not receive it (50% vs. 22%). The overall disease control (ORR + SD) frequency was 53%. We saw no difference in disease control rate by histology or the addition of bevacizumab. Median PFS was 3.3 months (95% CI 2.1–6.8 months; Fig. 2). Patients in Cohort 1 showed an apparent improved PFS compared to Cohort 2 (3.3 months vs. 2.9 months) but this was not statistically significant (p-value < 0.05).

Safety and tolerability

We considered all 40 patients (8 patients from phase Ib; 32 from phase II) who received one or more cycles on protocol as evaluable for toxicity. Most AE's were grade 2 or less and the most common included: neutropenia (35%), peripheral sensory neuropathy (32.5%), anemia (32.5%), fatigue (27.5%), weight loss (22.5%) and alopecia (17.5%) (Table 3). Thirteen patients experienced grade 3 or higher AE (2 on phase I; 11 on phase II) with the most common as anemia, neutropenia, dyspnea, fatigue and dehydration. There was one treatment-related death on phase II from pancytopenia and resulting sepsis.

Biomarker analysis

As prior preclinical data showed strong association between autophagy inhibition and arrest of tumor growth in *KRAS*-mutated tumors, we tested for *KRAS* status in the tumors of patients enrolled on this trial. Adequate archival tissue allowed us to check *KRAS* status in 16 patients, 9 of which tested positive for a *KRAS* mutation. In these 9 patients, ORR was 44% and disease control rate was 56%. The median PFS was higher than expected at 6.4 months (95% CI 1.8–15.6).

Discussion

The treatment of metastatic NSCLC continues to evolve rapidly. Antibodies directed against immune checkpoints such as PD-1 and PD-L1 are incorporated into part of the standard of care in first-line as well as second-line settings for treatment as monotherapy as well as in combination with chemotherapy. However, only a subset of patients respond to immunotherapy, and few experience complete responses. Therefore, novel combinations remain urgently needed to treat the majority of patients including those whose disease either fails to respond to immunotherapy combinations, those whose disease eventually progresses on these treatments. In this small phase Ib/II trial, we demonstrated that while the addition of the autophagy inhibitor, hydroxychloroquine to chemotherapy showed no improvement in progression-free survival, we, nevertheless, observed an small improvement in response rates compared to chemotherapy alone. Compared to prior studies of first-line chemotherapy in metastatic NSCLC [17,18] which reported ORR of 15–20% (and up to 35% if bevacizumab were added to regimen), we observed an ORR of 33% overall and 55% in patients who also received bevacizumab. We also established that 200 mg BID of hydroxychloroquine in combination with chemotherapy is safe and tolerable.

Unlike normal cells, tumor cells can have higher levels of basal autophagy, thereby making them constitutively dependent on autophagy for survival [9,19]. Up-regulation of autophagy in cancer cells is caused by direct activation of the transcription factors of the microphthalmia-associated transcription factor (MiTF)/TFE family that control autophagy and lysosomal biogenesis or by removal of a repressive phosphorylation on the autophagy initiation machinery [20,21]. Autophagy is also induced in hypoxic tumor regions and confers a survival advantage [19]. Experiments in genetically engineered mouse models (GEMMs) for cancer, where essential autophagy genes are ablated, show the functional importance of autophagy for various aspects of tumorigenesis [22]. Autophagy in these GEMMs for cancer promotes the growth, survival, and malignancy of a broad spectrum of cancers [23]. Moreover, deletion of an essential autophagy gene compromises the survival of those tumor cells in vitro and in tumors in vivo [9,24]. In lung cancer GEMMs, deletion of an essential autophagy gene *Atg7* dramatically alters tumor pathology from carcinomas to that of benign oncocytomas, which are human tumors that massively accumulate defective mitochondria [25,26]. These findings suggest that autophagy inhibition may compromise tumor survival and may be an effective

Table 2
Response rate in patients enrolled to phase II and evaluable for response (n = 30).

	All (n = 30)	By histology		By treatment arm		In *KRAS* + tumors (n = 9)
		Non-squamous (n = 17)	Squamous (n = 13)	Cohort 1 (n = 12)	Cohort 2 (n = 18)	
CR	0	0	0	0	0	0
PR	10 (33%)	6 (35%)	4 (31%)	6 (50%)	4 (22%)	4 (44%)
SD	6 (20%)	3 (18%)	3 (23%)	1 (8%)	5 (28%)	1 (11%)
Progressive disease	14 (47%)	8 (47%)	6 (44%)	5 (42%)	9 (50%)	4 (44%)
Disease control (CR/PR/SD)	16 (53%)	9 (53%)	7 (56%)	7 (58%)	9 (50%)	5 (56%)

Response rate represents best overall response rate (investigator).
CR, complete response; PR, partial response; SD, stable disease.

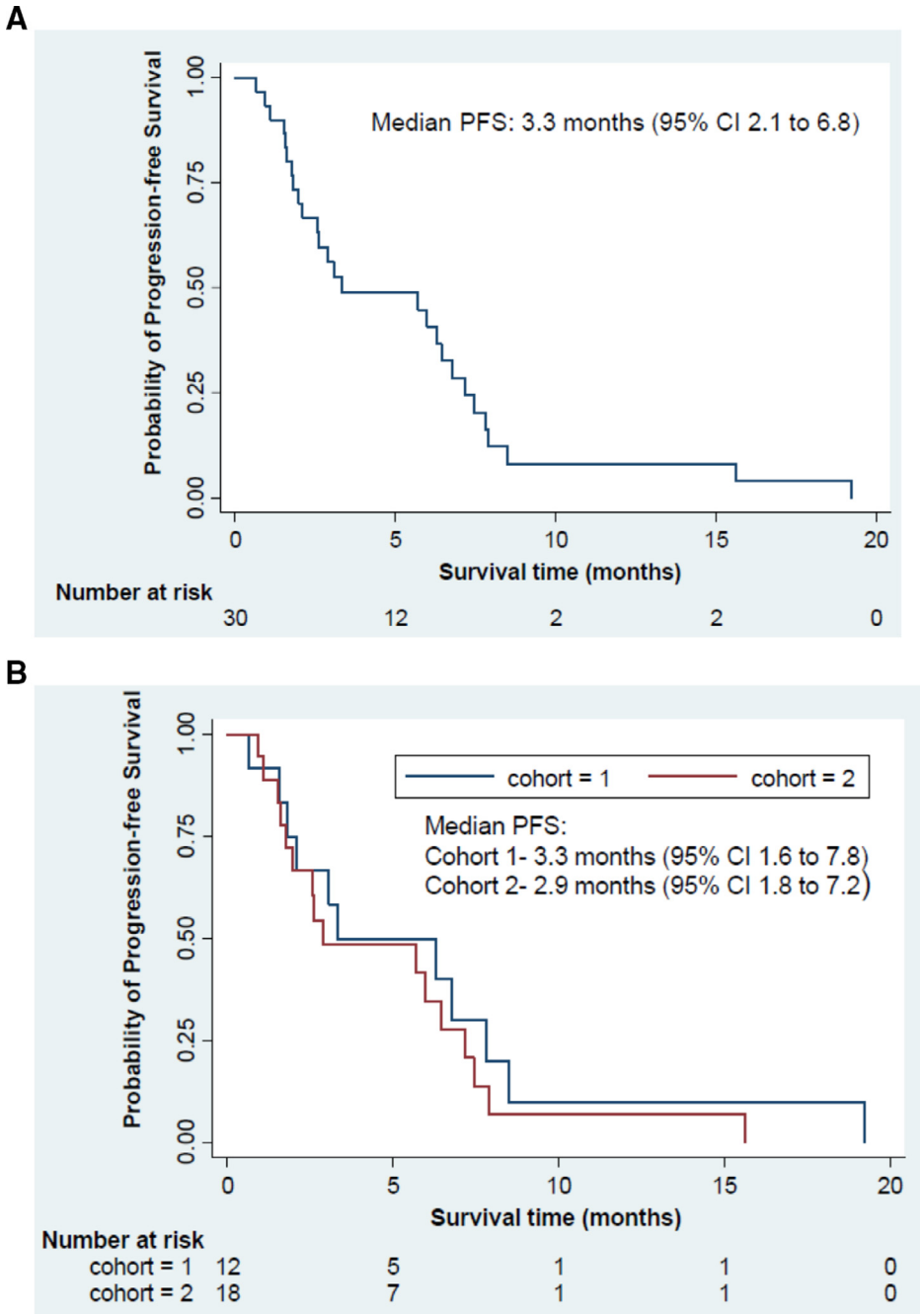

Fig. 2. Kaplan–Meier curves for progression-free survival (months).

Table 3
Selected treatment-related adverse events in all patients (n = 40; phase Ib and II).

Adverse Event		All grades		Grade ≥ 3	
		n	%	*n*	%
Hematological	Anemia	13	32.5	4	10
	Neutropenia	14	35	12	30
	Thrombocytopenia	5	12.5	2	5
Gastrointestinal	Abdominal pain	3	7.5	1	2.5
	Nausea	5	12.5	1	2.5
	Vomiting	4	10	0	0
	Diarrhea	5	12.5	0	0
General	Fatigue	11	27.5	4	10
	Anorexia	6	15	3	7.5
	Weight loss	9	22.5	0	0
	Dehydration	5	12.5	4	10
	Alopecia	7	17.5	0	0
	Rash	4	10	0	0
Respiratory	Bronchial infection	3	7.5	1	2.5
	Cough	4	10	1	2.5
	Dyspnea	6	15	3	7.5
Metabolism	Hyponatremia	3	7.5	2	5
Musculoskeletal	Generalized weakness	4	10	2	5
Nervous system	Peripheral sensory neuropathy	13	32.5	0	0

approach to target for cancer therapy [23].

Accumulating clinical evidence from other studies also suggests that inhibiting autophagy may constitute an efficient means to improve the therapeutic profile of chemo-, radio- and immunotherapeutic anticancer regimens [27]. A recent study shows that the combination of MEK inhibitor trametinib and hydroxychloroquine led to a striking disease response in a patient with pancreatic cancer [28]. O'Hara et al. reported the results from a phase II, single arm study in patients with metastatic colon cancer treated with hydroxychloroquine in combination with standard first-line chemotherapy (mFOLFOX6) [29]. In the 28 evaluable patients, the ORR was 68% with an 11% CR rate. The majority of patients showed an increase in both LC3 and p62 in peripheral blood mononuclear cells, and exhibited an increase in autophagosomes within the cytosol by electron microscopy. Another study treated patients with refractory metastatic colorectal cancer with a combination of hydroxychloroquine and vorinostat every three weeks [30]. In the 19 patients evaluable for survival, median PFS was 2.8 months and median overall survival was 6.7 months. Treatment-related grade 3 or higher AEs occurred in 8 patients (40%), with fatigue, nausea/vomiting, and anemia seen as the most common. On-study tumor biopsies showed increases in lysosomal protease cathepsin D and p62 accumulation, consistent with autophagy inhibition. Of note, there are few studies with reported safety outcomes evaluating hydroxychloroquine in combination with chemotherapy, and our study adds substantial experience with this combination. The vast majority of toxicity was attributable to hematological toxicities and neuropathy.

KRAS is the most common mutation found in NSCLC and unfortunately, no treatment exists that targets this mutation. Patients with KRAS-mutated lung cancer have worse prognosis and worse response rates to standard cancer treatments [31,32]. Autophagy, a catabolic pathway that involves the engulfment of macromolecules and certain cellular organelles, has been described as an adaptive response to KRAS-induced stress. Over the past several years, it has been shown that oncogenic RAS signaling triggers autophagic vacuole formation and that inhibition of autophagy by genetic or pharmacologic means can impair RAS-mediated transformation [9]. More recent studies in oncogenic KRAS-induced lung GEMMs with or without p53 have revealed that autophagy is required for mitochondrial function, lipid metabolism, growth, and fate of KRAS-driven lung tumors [25]. Moreover, acute, systemic genetic ablation of autophagy essential gene *Atg7* in mice with established K-ras$^{LSL\text{-}G12D/+}$;p53$^{fl/fl}$ (KP) NSCLC promotes tumor regression prior to damage to most normal tissues [12], suggesting that targeting autophagy or its downstream metabolic pathways is a potential therapy for the treatment of Kras-mutated lung cancer. In our study, we observed a higher ORR and PFS in this molecular subgroup compared to the overall ORR and PFS in the study. However, the number of patients with known *KRAS* mutated status in our study was small (n = 9).

Limitations of our study include small size and that it was not powered to detect a difference in PFS or overall survival. Our study was designed to treat patients with newly diagnosed stage IV NSCLC. However, the standard of care for first-line setting now includes chemotherapy in combination with PD-1/PD-L1 targeting immunotherapy such as pembrolizumab and our study was in immunotherapy-naïve patients. With the shift of immunotherapy from second-line to first –line setting, an unmet need arose for additional treatment options in patients who progress on initial combination therapy with chemotherapy and immunotherapy. While our trial was not performed in immunotherapy-resistant patients, our results do support further investigation of the combination of hydroxychloroquine with chemotherapy in immunotherapy-resistant patients.

Conclusion

The addition of hydroxychloroquine to chemotherapy was safe and resulted in modest improvement in response rate for select subgroup of patients with newly diagnosed metastatic NSCLC. Further studies investigating inhibition of this pathway in combination with chemotherapy and/or immunotherapy in NSCLC including specific genomic subgroup such as KRAS-mutated tumors appear warranted.

Declaration of Competing Interest

None.

References

[1] L.A. Torre, R.L. Siegel, A. Jemal, Lung cancer statistics, Adv. Exp. Med. Biol. 893 (2016) 1–19, https://doi.org/10.1007/978-3-319-24223-1_1 PubMed PMID: 26667336.
[2] G. D'Addario, M. Pintilie, N.B. Leighl, R. Feld, T. Cerny, F.A. Shepherd, Platinum-based versus non-platinum-based chemotherapy in advanced non-small-cell lung cancer: a meta-analysis of the published literature, J. Clin. Oncol. 23 (13) (2005) 2926–2936, https://doi.org/10.1200/JCO.2005.03.045 PubMed PMID: 15728229.
[3] K. Kelly, J. Crowley, P.A. Bunn Jr., C.A. Presant, P.K. Grevstad, C.M. Moinpour, S.D. Ramsey, A.J. Wozniak, G.R. Weiss, D.F. Moore, V.K. Israel, R.B. Livingston, D.R. Gandara, Randomized phase III trial of paclitaxel plus carboplatin versus vinorelbine plus cisplatin in the treatment of patients with advanced non–small-cell lung cancer: a Southwest Oncology Group trial, J. Clin. Oncol. 19 (13) (2001) 3210–3218, https://doi.org/10.1200/JCO.2001.19.13.3210 PubMed PMID: 11432888.
[4] B. Levine, D.J. Klionsky, Development by self-digestion: molecular mechanisms and biological functions of autophagy, Dev. Cell. 6 (4) (2004) 463–477 PubMed PMID: 15068787.
[5] K. Degenhardt, R. Mathew, B. Beaudoin, K. Bray, D. Anderson, G. Chen, C. Mukherjee, Y. Shi, C. Gelinas, Y. Fan, D.A. Nelson, S. Jin, E. White, Autophagy promotes tumor cell survival and restricts necrosis, inflammation, and tumorigenesis, Cancer Cell 10 (1) (2006) 51–64, https://doi.org/10.1016/j.ccr.2006.06.001 PubMed PMID: 16843265; PMCID: 2857533.
[6] V. Karantza-Wadsworth, S. Patel, O. Kravchuk, G. Chen, R. Mathew, S. Jin, E. White, Autophagy mitigates metabolic stress and genome damage in mammary tumorigenesis, Genes. Dev. 21 (13) (2007) 1621–1635, https://doi.org/10.1101/gad.1565707 PubMed PMID: 17606641; PMCID: 1899472.
[7] R. Mathew, V. Karantza-Wadsworth, E. White, Role of autophagy in cancer, Nat. Rev. Cancer 7 (12) (2007) 961–967, https://doi.org/10.1038/nrc2254 PubMed PMID: 17972889; PMCID: 2866167.
[8] A.C. Kimmelman, E. White, Autophagy and tumor metabolism, Cell. Metab. 25 (5) (2017) 1037–1043, https://doi.org/10.1016/j.cmet.2017.04.004 PubMed PMID: 28467923; PMCID: 5604466.
[9] J.Y. Guo, H.Y. Chen, R. Mathew, J. Fan, A.M. Strohecker, G. Karsli-Uzunbas, J.J. Kamphorst, G. Chen, J.M. Lemons, V. Karantza, H.A. Coller, R.S. Dipaola, C. Gelinas, J.D. Rabinowitz, E. White, Activated Ras requires autophagy to maintain oxidative metabolism and tumorigenesis, Genes. Dev. 25 (5) (2011) 460–470, https://doi.org/10.1101/gad.2016311 PubMed PMID: 21317241; PMCID: 3049287.
[10] J.Y. Guo, X. Teng, S.V. Laddha, S. Ma, S.C. Van Nostrand, Y. Yang, S. Khor, C.S. Chan, J.D. Rabinowitz, E. White, Autophagy provides metabolic substrates to

maintain energy charge and nucleotide pools in Ras-driven lung cancer cells, Genes. Dev. 30 (15) (2016) 1704–1717, https://doi.org/10.1101/gad.283416.116 PubMed PMID: 27516533; PMCID: 5002976.

[11] V. Bhatt, K. Khayati, Z.S. Hu, A. Lee, W. Kamran, X. Su, J.Y. Guo, Autophagy modulates lipid metabolism to maintain metabolic flexibility for Lkb1-deficient Kras-driven lung tumorigenesis, Genes. Dev. 33 (3–4) (2019) 150–165, https://doi.org/10.1101/gad.320481.118 Epub 2019/01/30PubMed PMID: 30692209.

[12] G. Karsli-Uzunbas, J.Y. Guo, S. Price, X. Teng, S.V. Laddha, S. Khor, N.Y. Kalaany, T. Jacks, C.S. Chan, J.D. Rabinowitz, E. White, Autophagy is required for glucose homeostasis and lung tumor maintenance, Cancer Discov 4 (8) (2014) 914–927, https://doi.org/10.1158/2159-8290.CD-14-0363 PubMed PMID: 24875857; PMCID: 4125614.

[13] B.M. Wolpin, D.A. Rubinson, X. Wang, J.A. Chan, J.M. Cleary, P.C. Enzinger, C.S. Fuchs, N.J. McCleary, J.A. Meyerhardt, K. Ng, D. Schrag, A.L. Sikora, B.A. Spicer, L. Killion, H. Mamon, A.C. Kimmelman, Phase II and pharmacodynamic study of autophagy inhibition using hydroxychloroquine in patients with metastatic pancreatic adenocarcinoma, Oncologist 19 (6) (2014) 637–638, https://doi.org/10.1634/theoncologist.2014-0086 PubMed PMID: 24821822; PMCID: 4041680.

[14] S.B. Goldberg, J.G. Supko, J.W. Neal, A. Muzikansky, S. Digumarthy, P. Fidias, J.S. Temel, R.S. Heist, A.T. Shaw, P.O. McCarthy, T.J. Lynch, S. Sharma, J.E. Settleman, L.V. Sequist, A phase I study of erlotinib and hydroxychloroquine in advanced non-small-cell lung cancer, J. Thorac. Oncol. 7 (10) (2012) 1602–1608, https://doi.org/10.1097/JTO.0b013e318262de4a PubMed PMID: 22878749; PMCID: 3791327.

[15] D.T. Vogl, E.A. Stadtmauer, K.S. Tan, D.F. Heitjan, L.E. Davis, L. Pontiggia, R. Rangwala, S. Piao, Y.C. Chang, E.C. Scott, T.M. Paul, C.W. Nichols, D.L. Porter, J. Kaplan, G. Mallon, J.E. Bradner, R.K. Amaravadi, Combined autophagy and proteasome inhibition: a phase 1 trial of hydroxychloroquine and bortezomib in patients with relapsed/refractory myeloma, Autophagy 10 (8) (2014) 1380–1390, https://doi.org/10.4161/auto.29264 PubMed PMID: 24991834; PMCID: 4203515.

[16] M.R. Rosenfeld, X. Ye, J.G. Supko, S. Desideri, S.A. Grossman, S. Brem, T. Mikkelson, D. Wang, Y.C. Chang, J. Hu, Q. McAfee, J. Fisher, A.B. Troxel, S. Piao, D.F. Heitjan, K.S. Tan, L. Pontiggia, P.J. O'Dwyer, L.E. Davis, R.K. Amaravadi, A phase I/II trial of hydroxychloroquine in conjunction with radiation therapy and concurrent and adjuvant temozolomide in patients with newly diagnosed glioblastoma multiforme, Autophagy 10 (8) (2014) 1359–1368, https://doi.org/10.4161/auto.28984 PubMed PMID: 24991840; PMCID: 4203513.

[17] A. Sandler, R. Gray, M.C. Perry, J. Brahmer, J.H. Schiller, A. Dowlati, R. Lilenbaum, D.H. Johnson, Paclitaxel-carboplatin alone or with bevacizumab for non-small-cell lung cancer, N. Engl. J. Med. 355 (24) (2006) 2542–2550, https://doi.org/10.1056/NEJMoa061884 PubMed PMID: 17167137.

[18] Y. Ohe, Y. Ohashi, K. Kubota, T. Tamura, K. Nakagawa, S. Negoro, Y. Nishiwaki, N. Saijo, Y. Ariyoshi, M. Fukuoka, Randomized phase III study of cisplatin plus irinotecan versus carboplatin plus paclitaxel, cisplatin plus gemcitabine, and cisplatin plus vinorelbine for advanced non-small-cell lung cancer: Four-Arm Cooperative Study in Japan, Ann. Oncol. 18 (2) (2007) 317–323, https://doi.org/10.1093/annonc/mdl377 PubMed PMID: 17079694.

[19] K. Degenhardt, G. Chen, T. Lindsten, E. White, BAX and bak mediate p53-independent suppression of tumorigenesis, Cancer Cell 2 (3) (2002) 193–203 PubMed PMID: 12242152.

[20] R.M. Perera, S. Stoykova, B.N. Nicolay, K.N. Ross, J. Fitamant, M. Boukhali, J. Lengrand, V. Deshpande, M.K. Selig, C.R. Ferrone, J. Settleman, G. Stephanopoulos, N.J. Dyson, R. Zoncu, S. Ramaswamy, W. Haas, N. Bardeesy, Transcriptional control of autophagy-lysosome function drives pancreatic cancer metabolism, Nature 524 (7565) (2015) 361–365, https://doi.org/10.1038/nature14587 PubMed PMID: 26168401; PMCID: 5086585.

[21] P.M. Wong, Y. Feng, J. Wang, R. Shi, X. Jiang, Regulation of autophagy by coordinated action of mTORC1 and protein phosphatase 2A, Nat. Commun. 6 (2015) 8048, https://doi.org/10.1038/ncomms9048 PubMed PMID: 26310906; PMCID: 4552084.

[22] R. Amaravadi, A.C. Kimmelman, E. White, Recent insights into the function of autophagy in cancer, Genes. Dev. 30 (17) (2016) 1913–1930, https://doi.org/10.1101/gad.287524.116 PubMed PMID: 27664235; PMCID: 5066235.

[23] R.K. Amaravadi, J. Lippincott-Schwartz, X.M. Yin, W.A. Weiss, N. Takebe, W. Timmer, R.S. DiPaola, M.T. Lotze, E. White, Principles and current strategies for targeting autophagy for cancer treatment, Clin. Cancer. Res. 17 (4) (2011) 654–666, https://doi.org/10.1158/1078-0432.CCR-10-2634 PubMed PMID: 21325294; PMCID: 3075808.

[24] S. Yang, X. Wang, G. Contino, M. Liesa, E. Sahin, H. Ying, A. Bause, Y. Li, J.M. Stommel, G. Dell'antonio, J. Mautner, G. Tonon, M. Haigis, O.S. Shirihai, C. Doglioni, N. Bardeesy, A.C. Kimmelman, Pancreatic cancers require autophagy for tumor growth, Genes. Dev. 25 (7) (2011) 717–729, https://doi.org/10.1101/gad.2016111 PubMed PMID: 21406549; PMCID: 3070934.

[25] J.Y. Guo, G. Karsli-Uzunbas, R. Mathew, S.C. Aisner, J.J. Kamphorst, A.M. Strohecker, G. Chen, S. Price, W. Lu, X. Teng, E. Snyder, U. Santanam, R.S. Dipaola, T. Jacks, J.D. Rabinowitz, E. White, Autophagy suppresses progression of K-ras-induced lung tumors to oncocytomas and maintains lipid homeostasis, Genes. Dev. 27 (13) (2013) 1447–1461, https://doi.org/10.1101/gad.219642.113 PubMed PMID: 23824538; PMCID: 3713426.

[26] A.M. Strohecker, J.Y. Guo, G. Karsli-Uzunbas, S.M. Price, G.J. Chen, R. Mathew, M. McMahon, E. White, Autophagy sustains mitochondrial glutamine metabolism and growth of brafV600E-driven lung tumors, Cancer Discov. 3 (11) (2013) 1272–1285, https://doi.org/10.1158/2159-8290.CD-13-0397 PubMed PMID: 23965987; PMCID: 3823822.

[27] C.G. Kinsey, S.A. Camolotto, A.M. Boespflug, K.P. Gullien, M. Foth, A. Truong, S.S. Schuman, J.E. Shea, M.T. Seipp, J.T. Yap, L.D. Burrell, D.H. Lum, J.R. Whisenant, G.W. Gilcrease 3rd, C.C. Cavalieri, K.M. Rehbein, S.L. Cutler, K.E. Affolter, A.L. Welm, B.E. Welm, C.L. Scaife, E.L. Snyder, M. McMahon, Protective autophagy elicited by RAF→MEK→ERK inhibition suggests a treatment strategy for RAS-driven cancers, Nat. Med. (2019), https://doi.org/10.1038/s41591-019-0367-9 Epub 2019/03/06PubMed PMID: 30833748.

[28] K.L. Bryant, C.A. Stalnecker, D. Zeitouni, J.E. Klomp, S. Peng, A.P. Tikunov, V. Gunda, M. Pierobon, A.M. Waters, S.D. George, G. Tomar, B. Papke, G.A. Hobbs, L. Yan, T.K. Hayes, J.N. Diehl, G.D. Goode, N.V. Chaika, Y. Wang, G.F. Zhang, A.K. Witkiewicz, E.S. Knudsen, E.F. Petricoin 3rd, P.K. Singh, J.M. Macdonald, N.L. Tran, C.A. Lyssiotis, H. Ying, A.C. Kimmelman, A.D. Cox, C.J. Der, Combination of ERK and autophagy inhibition as a treatment approach for pancreatic cancer, Nat. Med. 25 (4) (2019) 628–640, https://doi.org/10.1038/s41591-019-0368-8 PubMed PMID: 30833752; PMCID: 6484853.

[29] M. O'Hara, T. Karasic, I. Vasilevskaya, M. Redlinger, A. Loaiza-Bonilla, U. Teitelbaum, B. Giantonio, N. Damjanov, K. Reiss, M. Rosen, D.F. Heitjan, A.B. Troxel, R. Amaravadi, P.J. O'Dwyer, Phase II trial of the autophagy inhibitor hydroxychloroquine with FOLFOX and bevacizumab in front line treatment of metastatic colorectal cancer, J. Clin. Oncol. 35 (May (15_suppl)) (2017) 3545–3545.

[30] S. Patel, V. Hurez, S.T. Nawrocki, M. Goros, J. Michalek, J. Sarantopoulos, T. Curiel, D. Mahalingam, Vorinostat and hydroxychloroquine improve immunity and inhibit autophagy in metastatic colorectal cancer, Oncotarget 7 (37) (2016) 59087–59097, https://doi.org/10.18632/oncotarget.10824 PubMed PMID: 27463016; PMCID: 5312297.

[31] G. Metro, R. Chiari, C. Bennati, M. Cenci, B. Ricciuti, F. Puma, A. Flacco, A. Rebonato, D. Giannarelli, V. Ludovini, G. Bellezza, P. Ferolla, V. Minotti, L. Crino, Clinical outcome with platinum-based chemotherapy in patients with advanced nonsquamous EGFR wild-type non-small-cell lung cancer segregated according to KRAS mutation status, Clin. Lung. Cancer 15 (1) (2014) 86–92, https://doi.org/10.1016/j.cllc.2013.08.002 PubMed PMID: 24139827.

[32] D. Meng, M. Yuan, X. Li, L. Chen, J. Yang, X. Zhao, W. Ma, J. Xin, Prognostic value of K-RAS mutations in patients with non-small cell lung cancer: a systematic review with meta-analysis, Lung Cancer 81 (1) (2013) 1–10, https://doi.org/10.1016/j.lungcan.2013.03.019 PubMed PMID: 23608713.