Carcinomas of lung are the major causes of deaths due to cancers among men (244, 245). According to recent statistics, while about 1.8 million people are diagnosed with lung cancer, approximately 1.6 million individuals die due to lung cancers (31, 246, 247). Moreover, the 5-year survival rates vary significantly from 4–17% depending on stage of lung cancer and regional variations. All though new targeted therapies such as Portrazza (necitumumab), Tagrisso (osimertinib), and Alecensa (alectinib) have been given the approval by FDA for treating lung carcinomas, they are selective and work only for a sub-set of patients (247-250). For example, on November 24, 2015, the U.S. FDA granted approval to necitumumab (PORTRAZZA, Eli Lilly and Company), a recombinant human IgG1 monoclonal antibody that blocks the EGFR signaling, in combination with gemcitabine and cisplatin for first-line treatment of patients with metastatic squamous non-small cell lung cancer (NSCLC). But, necitumumab is not indicated for treatment of non-squamous NSCLC (251, 252). Likewise, approval was also given to Alecensa (alectinib) to treat individuals with advanced (metastatic) ALK anaplastic lymphoma kinase-positive non-small cell lung cancer (NSCLC) whose disease has worsened after, or who could not tolerate treatment with, another therapy called Xalkori (crizotinib) (253, 254). ALK gene mutation has been reported in 5% NSCLC lung cancers. Individuals with ALK-positive NSCLC show extensive metastasis in the brain (255-257). Therefore, the existing therapies are very selective and work only for individuals with respective target abnormalities. Hence, more effective therapies that work better in majority of the cases are urgently needed, which require identification of a good therapeutic target that is present and regulate the development of lung cancers, and whose modulation prevent or treat lung cancers more effectively.

Nrf2 is one such target in lung cancers (114, 129, 144, 258). However, increasing evidences have shown dual roles for the Keap1–Nrf2 pathway in lung tumors initiation and progression (69, 259). While one study has shown that the upregulation of Nrf2 is closely related to tumor protection, drug resistance and poor prognosis (35, 48), a separate study highlighted that Nrf2 act as a tumor suppressor by preventing cells from undergoing oncogenesis (259-261). Therefore, the role of Nrf2 (as a tumor suppressor or oncogene) depends on the stage of tumor progression. In this Ph.D. dissertation it is observed that the expression of Nrf2 is elevated in poorly differentiated metastatic lung carcinomas conferring the oncogenic role of Nrf2. Hence, it is likely that targeted inhibition of Nrf2 make the tumors more susceptible for cellular death. Providing experimental evidences, prior studies testing the efficacy of Nrf2 siRNAs for treating lung cancer cells A549 showed decreased cell proliferation rates and susceptibility to chemotherapeutic and radiation treatments upon inhibiting Nrf2 (48, 54, 95).

More over, targeting Nrf2 signaling using specific chemicals also showed tumor growth inhibitory effect (128, 262). Therefore, identifying naturally occurring therapeutic agents targeting Nrf2 signaling is highly significant and contribute to the development of better anti-lung cancer agents. In this study, a set of extracts collected from plants with proven anti-cancer activity were screened and identified Anacyclus pyrethrum and Glycyrrhiza glabra as potential candidates for inhibiting A549 lung cancer cells, which expresses a very high Nrf2. Even though the ethanolic extracts of AP and GG viz, APE and GGE, exhibited anti-cancer activity the selectivity is much better with APE as it has minimal effect on human fibroblast cell line FF2441. The anticancer potential of AP is not well reported, other than few
recent publications, in literature (263-265). Relatively, the anti-cancer potential of GG was more reported but not much is known about its efficacy for inhibiting lung cancers (266-268). Therefore, we have explored the anti-lung cancer potential of APE and GGE extracts by studying the (a) safety and efficacy in vitro and in vivo; and (b) molecular mechanisms, especially effect on Nrf2 signaling, by which these extracts are inhibiting cancer cells growth.

The data from this study shows that APE and GGE are safe to administer to mice and inhibit tumor cells proliferation (as evidenced by decreased body weight growth rate) by modulating the expression of Nrf2 pathway in a dose dependent manner. While doses lower than and higher than IC50 values inhibit Nrf2, a significant increase was observed in Nrf2 level at IC50 concentration (Figure 25). This dose dependent Nrf2 modulator effect of APE and GGE could be due to the presence of a mixture of compounds in these extracts causing this effect. For example, the elevated Nrf2 observed at IC50 concentration could be due to increased stress exerted by the treatment with extracts. It is well documented that oxidative stress induced in cells elevates Nrf2 to mitigate the ROS (58, 269, 270). The cell death observed due to APE and GGE at very high dose (higher than IC50) is a cumulative effect as these extracts also inhibit survival signaling (Akt signaling) and proliferation cascades (Cyclin D1) (271, 272). Dose dependent variations in Nrf2 signaling have been reported in many cancer cells treated with pharmacological agents(16, 273, 274). For instance, a study has shown that sulforaphane induces Nrf2 thereby protect cells from oxidative stress. However a separate investigation reported that sulforaphane inhibit cancer cells growth by targeting Nrf2 (275, 276). Similarly, anti-cancer agent curcumin is known to elevate Nrf2 in a dose dependent manner(277, 278). Therefore, it is important to first clarify the role of Nrf2 in lung cancer at different tumor stages, and then decide about the treatment.

Even though in vitro studies demonstrated the Nrf2 modulator effects of APE and GGE, in vivo no significant changes were observed in Nrf2 expression or activity (as evidenced by NQO1 activity assay). This could be due to (a) very high levels of Nrf2 expressed in EAC cells compared to even A549 lung cells; (b) or the compounds failed to inhibit the expression of Nrf2 in animals. However, the reduction in the weight gain (an indicator of tumor cells growth) observed with APE and GGE treatments is due to the effect of induction of apoptosis in EAC cells, which is probably mediated through the inhibition of survival kinases such as Akt. EAC cells are known to express high levels of Akt and targeted inhibition of Akt using phytochemicals from black tea are known to induce cell death (16, 279).

In summary, this study identified (a) Nrf2 as a marker for poorly differentiated lung carcinomas with metastatic ability; (b) APE and GGE as selective anti-cancer agents for inhibiting the growth of Nrf2 expressing human lung cancer cells A549 and mouse Ehrlich Ascites Carcinoma (EAC) cells; (c) dose dependent effects of APE and GGE on Nrf2 expression levels as well as on survival and proliferation marker proteins Akt and Cyclin-D1. However, more detailed studies are warranted to test the APE and GGE extracts on lung cancer cell lines expressing or not expressing Nrf2 to demonstrate whether these extracts are inhibiting the growth by inhibiting Nrf2 or by working through other mechanisms. Many Nrf2 inhibitors such as brusatol, a quassinoid isolated from the Brucea javanica shrub, and luteolin (a flavonoid present in broccoli, parsley and peppers) have been identified as unique inhibitors of Nrf2 pathway with known anti-cancer effects. For example, treatment with brusatol sensitized cancer cells to the antitumor drugs, reduced tumor burden, and improved survival in xenograft models. Likewise, treatment with luteolin significantly enhanced the antitumor efficacy of chemotherapeutic agents oxaliplat, doxorubicin, and bleomycin on A549 cells. The alkaloid trigonelline is another Nrf2 inhibitor with
antitumor activity. Treatment of trigonelline combined with etoposide enhanced the antitumor efficacy of etoposide and reduced tumor size (94, 280, 281). However, these Nrf2 inhibitors have not been evaluated in clinical trials. Probably reasons for not evaluating these inhibitors in clinical trials are (a) lack of sufficient preclinical data; (b) failure of these compounds when tested in higher animals and (c) lack of specificity and off target effects causing toxicity. Therefore, search for identifying better anti-Nrf2 agents for treating cancers still continues. APE and GGE might provide better anti-Nrf2 agents, but require further studies.