

# Lung cancer • 1: Prevention of lung cancer

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*Thorax* 2002;**57**:994–999

Cancer of the lung causes more deaths from cancer worldwide than at any other site. The environmental, genetic, and dietary risk factors are discussed and progress in chemoprevention is reviewed. A better understanding of the molecular events that occur during carcinogenesis has opened up new areas of research in cancer prevention and a number of biochemical markers of high risk individuals have been identified. It is predicted that greater success in chemoprevention will be achieved in the next decade than in the last.

## THE PROBLEM

In 1990 cancer caused an estimated six million deaths worldwide and, of these, lung cancer was the most frequent site with an estimated 945 000 deaths.<sup>1</sup> In 2002 the death rate from lung cancer in the USA in both men and women is estimated at 134 900, exceeding the combined total for breast, prostate, and colon cancer.<sup>2</sup> Lung cancer is also the leading cause of cancer death in all European countries and is rapidly increasing in the developing world. Of 40 countries worldwide, the countries with the highest rates are Hungary (81.6/100 000 person years), the Czech Republic, the Russian Federation, Poland, and Denmark.<sup>3</sup> Among women the highest rates were in the US (25.6/100 000 person years), Denmark, Canada, the UK, and New Zealand.

## ENVIRONMENTAL RISK FACTORS

In addition to being the biggest cancer killer, lung cancer is one of the few cancers with a well defined aetiology—namely, the inhalation of tobacco smoke. In the USA the death rate from lung cancer parallels the 1965 peak and subsequent decline in cigarette smoking rates. The incidence of lung cancer peaked in 1990 with 41.4 deaths per 1000 person years and has since fallen, reaching a rate of 39.8 in 1995.<sup>4</sup> Shopland *et al*<sup>5</sup> determined the prevalence of smoking in each of the 50 states from surveys conducted in 1992 and compared these rates with 1985. Kentucky and West Virginia were the highest rates with 32.29% and 30.59% of the adult population 20 years or older being current smokers. Utah had the lowest rate at 17.1%. Forty nine of the 51 areas had a decrease in smoking between 1985 and 1992–93. Rhode Island experienced the greatest decline at –30.7%. Only Utah (+16.3%) and Wisconsin (+1.9%) showed increases. Nationally, the prevalence of smoking declined 17% overall from 1985 to 1992–93. Among subgroups, African American men experienced the highest rates (31.3%) followed by White men (26.4%) and Hispanic

men (25.0%). Among women, Whites and African Americans were similar (22.9% and 22.5%, respectively) whereas smoking among Hispanic women was significantly less (12.7%).

While there have been similar declines in the incidence of smoking in Canada and western Europe, there is concern about the rising rates of smoking in developing countries.<sup>6–8</sup> China, the world's most populous country, has tripled cigarette consumption between 1978 and 1987.<sup>9</sup> It is estimated that 70% of Chinese men and 2% of women smoke.<sup>10</sup> Given the 20–30 year lag between exposure and peak incidence of cancer, the potential coming epidemic stresses the urgent need to develop effective prevention strategies.

In addition to the hazard of first hand smoke, the 1986 US Surgeon General's report concluded that cigarette smoke was a health risk to non-smokers. This has subsequently been supported by over 24 studies showing that exposed non-smokers have an increased relative risk of developing cancer ranging from 1.41 to 2.01.<sup>11–14</sup>

Clearly, cigarette smoking remains the most prevalent and uncontrolled environmental carcinogen in our society. The continued burden of current and ex-smokers in the US and western Europe (the majority of Americans developing lung cancer are ex-smokers<sup>15–17</sup>), the increasing incidence of smokers in Asia, and the recruitment of new smokers worldwide guarantee that lung cancer will remain the major cause of cancer death worldwide for the next 25–50 years.

Over the past 10 years there have been advances in our understanding of the epidemiology and genetics of nicotine addiction.<sup>18</sup> These findings have opened new areas of smoking prevention and cessation research using pharmacological interventions with both pharmacological agents and nicotine replacement.<sup>19</sup> As health professionals concerned about lung cancer, we must vigorously support smoking prevention and cessation programmes.<sup>20</sup> We must champion efforts to make tobacco abuse a socially and culturally unacceptable habit and support all governmental actions<sup>21</sup> to eliminate tobacco as an environmental carcinogen.

## GENETIC RISK FACTORS

Not all cigarette smokers go on to develop lung cancer. Investigators are working to identify factors which can predict individual susceptibility.<sup>22</sup> One area of study is the family of enzymes responsible for carcinogen activation, degradation, and subsequent DNA repair.<sup>23</sup> These enzymes display gene deletions and polymorphisms which can affect enzyme activity. It has been hypothesised that an individual's enzyme profile is associated with lung cancer risk. This profile could be used to counsel individuals and

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could be used to select high risk individuals for specific chemoprevention agents. These enzymes and the metabolic pathways they regulate also have the potential to become targets for preventive agents.

To illustrate this point, benzo[a]pyrene, one of the many carcinogens found in cigarette smoke, is metabolically activated by the P450 family of hepatic enzymes (mainly CYP1A1).<sup>24–27</sup> The intermediate metabolites are chemically active and can bind to DNA and cause gene dysfunction. Glutathione-S-transferase (GST), epoxide hydrolase (EH) and N-acetyltransferase (NAT) detoxify these products. Polymorphisms and/or gene deletions result in modified metabolic activity.<sup>18, 28–32</sup> Enzymes responsible for DNA repair also display polymorphisms.<sup>33–35</sup> Studies suggest that genetic alterations in each of these enzyme families can have small effects on an individual's risk of developing lung cancer.

Gene-diet interaction will also require careful investigation. Studies suggest that low levels of vitamin E can increase the GSTM1 associated risk.<sup>36</sup> Interactions with dietary enzyme factors such as folate and subsequent folate metabolism have also been suggested.<sup>37</sup> Although it is possible that a single polymorphism or dietary interactions may significantly alter the relative risk, it is likely that many interactions, each having a subtle effect, can result in synergistic interactions that greatly affect the overall risk. Determining the risk profile of an individual based on their inherited polymorphisms and their potential dietary interaction will be a complex undertaking. Testing these hypotheses will require studies with a very large sample size to achieve the statistical power.

### DIETARY RISK FACTORS

Numerous studies have shown that the incidence of cancer can be inversely related to the intake of many food groups.<sup>38–41</sup> The serum concentration of many micronutrients is also inversely related to the incidence of cancer.<sup>42–46</sup> Based on these epidemiological studies, it has been suggested that micronutrients and macronutrients present in our diet may act as cancer inhibiting substances.

Dietary carotenoids were one of the first micronutrients suggested as risk factors for lung cancer.<sup>38</sup> Epidemiological studies reported that individuals with a diet low in  $\beta$ -carotene-rich foods had a higher incidence of lung cancer.<sup>39, 40, 47, 48</sup> Retrospective case-control trials of serum obtained from individuals who later developed lung cancer confirmed that the serum concentrations of  $\beta$ -carotene were lower in cases than in controls.<sup>43, 48</sup> Those in the highest category had a relative risk of 0.5–0.7 compared with those in the highest. Another carotenoid, lycopene, a simple hydrocarbon precursor of  $\beta$ -carotene, has been studied.<sup>49</sup> Lycopene is an effective antioxidant (25% better than  $\beta$ -carotene) and is the second most common dietary carotenoid. The most common source of lycopene in the diet is cooked or processed tomatoes which contain about 30 mg/kg. Like  $\beta$ -carotene, epidemiological studies of the dietary intake or serum concentration of lycopene found an inverse relationship with cancer of the bladder, lung and prostate.<sup>49, 50</sup>

Other dietary micronutrients may also be associated with lung cancer risk. Knekt *et al* reported that dietary flavonoids (found in high concentration in apples) were a strong predictor of lung cancer risk. In a population of 9959 Finnish men and women, those with the highest intake of dietary flavonoids had an incidence of lung cancer that was 59% of those in the lowest quartile.<sup>51</sup> Isothiocyanates, which are widely distributed in cruciferous vegetables, have also been shown to have an inverse relationship with the incidence of lung cancer.<sup>52, 53</sup> In vivo animal model systems have shown that isothiocyanates have activity in decreasing the incidence of cancer of the lung, oesophagus, liver, colon, and bladder.

### CHEMOPREVENTION

The pragmatic acceptance that tobacco abuse cannot be easily and rapidly eliminated has given emphasis to the field of lung

cancer chemoprevention. Chemoprevention is defined as the use of agents to prevent, inhibit, or reverse the process of carcinogenesis.<sup>54</sup> The underlying hypothesis of prevention is that carcinogenesis is the stepwise accumulation of genetic and epigenetic changes that result in a cell with a malignant phenotype. The goal of cancer prevention scientists is to develop interventions that can interrupt, arrest, or reverse this process.<sup>55</sup>

Historically, two major categories of compounds have been investigated for cancer prevention activity. One group consists of naturally occurring dietary micronutrients and their synthetic analogues which have been discussed above. Although epidemiological associations cannot prove a cause and effect relationship, they show strong associations and suggest hypotheses to be tested. The goal is to determine which, if any, of these dietary substances (or combination of substances) are important factors in modifying the incidence of cancer,<sup>56</sup> and whether supplementation of the diet with these micronutrients is an effective method of cancer prevention.

The other group of compounds currently being investigated are synthetic agents.<sup>57</sup> These include a large number of compounds with varying mechanisms including, for example, the non-steroidal anti-inflammatory agents (NSAIDs) which are potent in vivo inhibitors of colon carcinogenesis<sup>58–60</sup> and agents such as DFMO (difluoromethyl ornithine), a polyamine synthase inhibitor, which has a broad spectrum of preventive activity in vitro and in vivo.<sup>61–63</sup>

### Retinoids

Vitamin A and its family of compounds (the retinoids) were the first dietary constituents to have extensive in vitro and in vivo evidence of chemopreventive activity.<sup>64</sup> The retinoids have been found to be active in many animal model systems using different organ sites as well as different inducing carcinogens.<sup>65</sup> When Sporn *et al* first discussed the concept of chemoprevention, his work focused on the retinoids.<sup>54</sup>

Retinol, its palmitic acid ester, the trace retinoids all-trans-retinoic acid and 13-cis-retinoic acid, together with the synthetic retinoids etretinate and 4-hydroxy phenyl retinamide have all been studied in vitro as well as in human intervention trials. Trials with these agents were started in the early 1980s and a number of them have matured and reported results.

One of the earliest reported positive trials was that by Hong *et al* who studied the effects of 13-cis-retinoic acid on the incidence of recurrence and second primaries in patients with each stage primary squamous cell carcinomas of the head and neck.<sup>66</sup> This tobacco-associated malignancy has many analogies to lung cancer. Although there was no decrease in the incidence of recurrence, second primaries which, in general, consisted of other tobacco related cancers (head and neck, oesophageal, and lung) were significantly fewer ( $p < 0.05$ ). This study is now undergoing confirmation in a large intergroup trial being conducted within the USA.

The European Organization for Cancer (EORTC) studied a population of patients with resected stage I non-small cell lung cancer. Like patients with early primary cancers of the head and neck, this group has a 60–70% cure rate of their primary cancer with surgery alone. Retinyl palmitate (300 000 IU/day) plus *N*-acetylcysteine (600 g/day) were given for two years. Their end points were recurrence and second primaries. This trial reported some early encouraging results although a final report in 2000 reported no benefit in survival, relapse free survival, or incidence of second primaries.<sup>67</sup>

A US intergroup trial of similar design tested 13-cis-retinoic acid (30 mg/day) for 3 years in a similar population of subjects with stage I lung cancer. This phase III trial completed accrual in 1997 and reported an increase in death rate in patients receiving 13-cis-retinoic acid. After a median follow up of 3.5

years there was no effect of the supplements on the time to second primaries, recurrence, or mortality. There was a smoking-treatment interaction, with current smokers on the active arm having increased recurrence and mortality.<sup>68</sup>

13-cis-retinoic acid has also been tested using bronchial metaplasia as the end point.<sup>69</sup> This double blind, randomised trial showed no decrease in the incidence of metaplasia in the active treatment arm. The positive results of the clinical trial in head and neck cancers using cancer as an end point and these negative results help to emphasise that intermediate end points need to be clearly linked to cancer incidence before they can be acceptable as a surrogate end point.

The retinoids as a family continue to be investigated for chemoprevention. It is likely that additional synthetic retinoids which block or activate specific retinoid receptors (RXR) will be developed and tested in the clinic.<sup>70</sup> Lung cancer will remain a prime target for these agents.

### Beta-carotene

Epidemiological trials, together with an influential science editorial in 1981, suggested that  $\beta$ -carotene was the most promising lung cancer prevention agent.<sup>6</sup> In spite of only limited in vitro and in vivo animal studies suggesting that  $\beta$ -carotene had preventive activity, human intervention trials were started in the early 1980s. Two of these trials focused on lung cancer—the Alpha Tocopherol Beta Carotene Trial (ATBC) in Finland<sup>71</sup> and the Carotene and Retinol Efficacy Trial (CARET) in the USA.<sup>72</sup>

CARET tested the combination of 30 mg  $\beta$ -carotene and 25 000 IU retinyl palmitate daily in 18 314 men and women aged 50–69 years at high risk for developing lung cancer.<sup>73</sup> The high risk groups consisted of 14 254 cigarette smokers with a 20-pack year smoking history, either current smokers or ex-smokers who had quit within 6 years, and 4060 men with extensive occupational asbestos exposure who were current or ex-smokers (up to 15 years since quitting). The intervention was stopped 21 months early because of evidence of no benefit and possible harm (mean follow up 4 years). There were 28% more lung cancers ( $p=0.02$ ) and 17% more deaths ( $p=0.02$ ) in the active intervention group. Because CARET administered a combination of  $\beta$ -carotene and retinyl palmitate it was not possible to distinguish whether the adverse effects were due to  $\beta$ -carotene, retinyl palmitate, or the combination.

These results were remarkably similar to the ATBC trial which was completed in Finland in a similar high risk population and reported in 1994, before CARET.<sup>74</sup> This NCI sponsored 2  $\times$  2 placebo controlled trial administered 20 mg  $\beta$ -carotene with or without 50 mg  $\alpha$ -tocopherol for 5–8 years (mean 6.1 years) to 29 133 Finnish men aged 50–69 who smoked five or more cigarettes daily. There was no overall effect of  $\alpha$ -tocopherol on the incidence of lung cancer (relative risk (RR) 0.99, 95% confidence interval (CI) 0.87 to 1.13,  $p=0.86$ ). On the other hand,  $\beta$ -carotene supplementation was associated with an increase in lung cancer risk (RR 1.16, 95% CI 1.02 to 1.33,  $p=0.02$ ). The adverse effect of  $\beta$ -carotene appeared to be stronger in those who were heavy smokers of at least 20 cigarettes per day (RR 1.25, 95% CI 1.07 to 1.46) than in those who smoked 19 cigarettes or less per day (RR 0.97, 95% CI 0.76 to 1.23). These two trials clearly established that  $\beta$ -carotene supplements are harmful to cigarette smokers causing an increase in the incidence of lung cancer and mortality.

Another smaller intervention trial has reported encouraging results in lung and other cancer. It has long been known that there is an inverse relationship between the incidence of cancer and the selenium content of the soil (and hence locally grown foods) and cancer.<sup>75</sup> Based on this finding, Clark *et al*<sup>76</sup> conducted a skin cancer prevention trial in individuals previously diagnosed as having skin cancer. Participants were randomised to receive 200  $\mu$ g/day selenium rich brewer yeast or placebo. An analysis in 1997 reported that selenium supplementation did not change the primary end point of new skin

cancers but did reduce the incidence of other primaries (lung,  $p=0.05$ ; prostate,  $p=0.001$ ; colorectal,  $p=0.03$ ). This study will require confirmation since the population consisted of patients with previous skin cancer and the encouraging results were seen only in the secondary analysis. A primary intervention trial of selenium and  $\alpha$ -tocopherol with a sample size of 32 000 with the primary end point of prostate cancer and secondary end points of lung cancer and colon cancer (SELECT) began recruitment in 2001.

The findings of CARET and ATBC were a surprise since they conflicted with the epidemiological data. However, both CARET and ATBC administered high doses of  $\beta$ -carotene (20–30 times the average daily intake). These results emphasise the importance of carefully controlled intervention trials in determining the role of dietary supplements or any intervention agent. Because of the discouraging results of the large intervention trials as well as a rapidly expanding understanding of lung cancer, there has been a shift in focus to small clinical trials evaluating the effect of potential intervention agents on biomarkers of carcinogenesis.

### BIOMARKERS OF CARCINOGENESIS

A better understanding of the molecular events that occur during carcinogenesis has opened new areas of research in cancer prevention. Currently, pre-neoplasia is diagnosed based on histological examinations. However, for lung cancer, bronchial metaplasia or dysplasia is not always a good predictor of future cancer risk. Ex-smokers can have improvement in metaplasia/dysplasia but their cancer risk remains increased.<sup>77–79</sup> It is hoped that testing bronchial mucosa or bronchial epithelial cells for the presence of genetic or epigenetic changes will better predict the risk of cancer. Patients with documented changes may be more appropriate for treatment with chemoprevention agents. In addition, chemoprevention trials can be based on these molecular markers. Patients can be recruited to a trial by the presence of one or more of these markers. Those agents which cause an improvement in marker profile can then be further investigated for cancer prevention activity. These scenarios remain hypotheses to be tested since none of the molecular markers described has been shown to be a reliable predictor of cancer incidence. Their natural history in high risk populations is unknown, and it is also unknown if an agent which causes an improvement in marker status will ultimately decrease the incidence of cancer.

Numerous candidate markers are being investigated. Alterations in the p53 tumour suppressor gene are commonly acquired genetic lesions observed in lung cancer.<sup>80–82</sup> Mutations affect approximately 90% of small cell lung cancers and 50–60% of non-small cell lung cancers.<sup>83–91</sup> Lonardo *et al*<sup>92</sup> studied the expression of p53 in bronchial epithelium and squamous cell carcinomas and found that 61% of the squamous cell carcinomas, 54% of high grade dysplasias, and only 6% with atypia overexpressed p53. p53 expression was not seen in squamous metaplasia or low grade dysplasia. Rusch *et al*<sup>93</sup> had similar findings with 56% of non-small cell and 16% of bronchial lesions exhibiting aberrant p53 immunohistochemical staining.

Mao *et al*<sup>94</sup> studied the sputum of high risk individuals and followed them for cancer incidence. They found that 10 of 15 patients who developed adenocarcinoma had mutations in the ras or p53 gene. They then examined sputum collected prior to diagnosis and found that eight of the 10 patients had the identical mutation detected in at least one sputum sample. Mao *et al*<sup>95</sup> later reported the detection of abnormalities in bronchoscopic epithelial biopsy specimens in 40 current smokers and 14 former smokers. They determined DNA sequence losses involving microsatellite DNA at three loci (3p14, 9p21, and 17p13). DNA losses at 3p14, 9p21, and 17p13 were detected in 27 (75%), 21 (57%), and six (18%), respectively, of the informative subjects (those who could be

evaluated at that specific loci). Only one abnormality, loss of 3p14, was seen in five of nine of the informative non-smokers. These sites of DNA loss are known to be localised to chromosomal sites important in cancer. The tumour suppressor gene p53 is located at 17p13.<sup>96</sup> The other two loci contain the tumour suppressor genes FHIT (3p14) and p16 (9p21).<sup>97-99</sup> This study showed that relevant genetic abnormalities can be seen in histologically normal bronchial epithelial cells of smokers and are compatible with the hypothesis of a stepwise accumulation of genetic abnormalities leading to cancer.

Inactivation of the p16 tumour suppressor gene resulting from either allelic loss or mutation of p16 or hypermethylation of CpG islands in its promoter region<sup>100</sup> is also a promising marker in lung cancer.<sup>101-103</sup> Belinsky's group used methylation-specific polymerase chain reaction (MSP)<sup>104-105</sup> to determine the frequency of p16 methylation in premalignant lesions, carcinoma in situ lesions, squamous cell carcinomas, and sputum samples. The frequency increased during disease progression from basal cell hyperplasia (17%) to squamous metaplasia (24%) to carcinoma in situ (50%) lesions. He further showed that aberrant p16 methylation could be detected in sputum samples from three of seven patients with lung cancer and five of 26 cancer-free individuals at high risk (smokers). Ahrendt<sup>101</sup> found methylated p16 alleles in prospectively collected bronchoalveolar lavage (BAL) fluid from 63% of patients (12/19) with a primary resectable non-small cell lung cancer that had p16 methylation in the cancer. Palmisano *et al*<sup>106</sup> found that aberrant methylation of the p16 and/or O6-methyl-guanine-DNA methyltransferase (MGMT) promoter regions can be detected in DNA from sputum in all 10 patients with squamous cell lung carcinoma at the time of diagnosis and also in sputum samples of all 11 patients up to 3 years before clinical diagnosis. Only five of these 21 patients had sputum cytologies positive for cancer. Furthermore, methylated p16 and MGMT sputum markers were detected in 12–19% and 16–36%, respectively, of cancer-free individuals at high risk (exposure to tobacco and/or radon) for developing lung tumours.

The cyclins are a family of nuclear factors that are expressed and control the progression of the cell through the cell cycle. Inappropriate expression or activity has been found in cancer and it has been suggested that the expression of these proteins may be a marker of early carcinogenesis. Lonardo *et al*<sup>92</sup> studied cyclin D1 and E in a series of bronchial biopsies ranging from metaplasia to low grade dysplasia, high grade dysplasia, and squamous cell carcinoma. They found that cyclin D1 was detected in 7% with squamous metaplasia, 15% with atypia, 18% with low grade dysplasia, 47% with high grade dysplasia, and 42% with squamous cell carcinoma. Findings were similar with cyclin E which was not detected in normal epithelium, squamous metaplasia, or low grade dysplasia but occurred in 9% with atypia, 33% with high grade dysplasia, and 54% with squamous cell carcinomas. Papadimitrakopoulou *et al*<sup>107</sup> studied 27 patients with biopsy specimens of the upper aerodigestive tract and found 50% expression of cyclin D.

Cyclo-oxygenase (COX) is a constitutively expressed enzyme which is one of the rate limited steps in the conversion of arachadonic acid to prostaglandins. An alternative form of COX, COX-2, is inducible and is expressed in response to growth factors and to other stimuli. It appears that COX-2 is expressed in many lung cancer cell line model systems and tumours obtained from patients. Overexpression of COX-2 occurs in all cell types but appears more prominent in well differentiated cancers. It is also found in premalignant stages and invasive cancers.<sup>108-112</sup> Many COX-2 inhibitors have inhibitory effects on cell lines.<sup>113-114</sup> It has been proposed that the expression of COX-2 may be an early marker of a genetically altered epithelial cell destined to become cancer. Wolff *et al*<sup>111</sup> found expression of COX-2 in 19 of 21 adenocarcinomas. Well differentiated adenocarcinomas appeared to have more COX-2 staining than poorly differentiated tumours. Expression of COX-2 was also seen in 11 squamous cell carcinomas.

Epidermal growth factor receptor (EGFR) is a member of the erbB gene family of transmembrane tyrosine kinase. Activation plays an important role in cell division and differentiation. Abnormalities of EGFR have been found in patients with lung cancer and those at high risk.<sup>115-118</sup> While EGFR is expressed in normal epithelium, increased expression has been in found metaplastic lesions<sup>119-120</sup> and in 70% of squamous cell carcinomas and around 50% of adenocarcinomas.

These markers are just a few of the many which have been described that may serve as intermediate end points in identifying high risk individuals. Currently, many are acting as end points for an intervention trial. Although each marker is firmly established as important in the carcinogenesis of lung cancer, modulation of these markers by preventive agents does not guarantee that the agent will have activity in reducing the incidence of lung cancer. Trials are currently being conducted to evaluate multiple markers.

A better understanding of carcinogenesis has also fostered the development of agents specifically targeting the epigenetic and genetic changes which develop in neoplastic cancer cells. Specific agents are now available that block the COX-2 enzyme (Celecoxib). EGFR is activated in lung cancer and is expressed in pre-neoplasia. ZD1839 is a specific inhibitor of the EGF activated tyrosine kinase. These compounds are examples of agents currently being tested in the treatment of patients with established lung cancer and have good potential as preventive agents.

Chemoprevention is a new field. Analogous to the development of cancer chemotherapy in the 1940s and 1950s, this field is in its infancy. As we gain a better understanding of carcinogenesis our development and use of agents will become based in modifying underlying mechanisms. Although the past 10–15 years have been disappointing, we have established a strong foundation for future clinical trials. There is every reason to believe that greater success will be achieved in the next 10–15 years.

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