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Review

Vaping and lung cancer – A review of current data and recommendations

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ABSTRACT

Objectives: Lung cancer is the most common cause of cancer mortality worldwide and, while tobacco smoke remains the primary cause, there is increasing concern that vaping and E-cigarette use may also increase lung cancer risk. This review concentrates on the current data, scholarship and active foci of research regarding potential cancer risk and oncogenic mechanisms of vaping and lung cancer.

Materials and methods: We performed a literature review of current and historical publications on lung cancer oncogenesis, vaping device/e-liquid contents and daughter products, molecular oncogenic mechanisms and the fundamental, potentially oncogenic, effects of electronic cigarette smoke/e-liquid products.

Results: E-cigarette devices and vaping fluids demonstrably contain a series of both definite and probable oncogens including nicotine derivatives (e.g. nitrosnornicotine, nitrosamine ketone), polycyclic aromatic hydrocarbons, heavy metals (including organometal compounds) and aldehydes/other complex organic compounds. These arise both as constituents of the e-liquid (with many aldehydes and other complex organics used as flavourings) and as a result of pyrolysis/complex organic reactions in the electronic cigarette device (including unequivocal carcinogens such as formaldehyde – formed from pyrolysis of glycerol). Various studies demonstrate *in vitro* transforming and cytotoxic activity of these derivatives. E-cigarette device use has been significantly increasing – particularly amongst the younger cohort and non-smokers; thus, this is an area of significant concern for the future.

Conclusion: Although research remains somewhat equivocal, there is clear reason for concern regarding the potential oncogenicity of E-Cigarettes/E-Liquids with a strong basic and molecular science basis. Given lag times (extrapolating from tobacco smoke data) of perhaps 20 years, this may have significant future public health implications. Thus, the authors feel further study in this field is strongly warranted and consideration should be made for tighter control and regulation of these products.

1. Introduction

The aetiology of lung cancer (LCa) represents perhaps the first successful application of epidemiologic principles to cancer risk - with the Doll and Hill studies establishing smoking as the primary risk factor for the disease [1,2]. Given the overall poor prognosis and lack of an easily detectable pre-malignant lesion in LCa, the focus has, understandably, been upon risk reduction – i.e. smoking cessation, particularly given its simultaneous benefits in other pathologies. Overall, since the report of US Surgeon General, Luther L Terry, in 1964 concluding that smoking was a definite cause of LCa in men and a probable cause of LCa in women, a concerted effort to reduce smoking rates has seen great strides and a progressive fall in smoking rates [3]. While smoking is and remains the primary cause of LCa, other causes (including radiation

Abbreviations: ALCa, lung adenocarcinoma; AYA, adolescent and young adult; DNA, deoxyribonucleic acid; EC, electronic cigarette/s; ECS, electronic cigarette smoke; EL, E-liquid; EMT, epithelial-mesenchymal transition; GRAS, generally regarded as safe; LCa, lung cancer; SLCa, squamous-cell lung cancer; TS, tobacco smoke; (US) FDA, (US) Food and Drug Administration; VL, vaping liquid.

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exposure, pulmonary fibrosis, radon gas, heavy metals etc) exist. Alternative strategies are necessary where non-smoking related LCa is concerned – e.g. radon gas may be both predicted geologically and directly measured, heavy metal exposure is frequently occupational or related to industrial contamination and pulmonary fibrosis and other chronic lung diseases frequently undergo regular medical surveillance. Notably, while cigarette-smoking rates have fallen total LCa rates have actually increased over time [4].

In light of both the addictive properties of nicotine and the potential habituation to the finger-mouth motion of cigarettes/cigars, both nicotine replacement therapy and devices to simulate the activity of smoking (i.e. inhalers) have become increasingly popular for the facilitation of smoking cessation. Nicotine replacement has demonstrated an unequivocal improvement in smoking cessation vs control across various trials – as demonstrated by Hartmann-Boyce et al. in a Cochrane review [5]. Ultimately, this has led to the present vaping devices – based on earlier cigarette-simulacrum inhalers and the vaping device produced by Chinese pharmacist Hon Lik – which vaporise a nicotine solution by means of an ultrasonic vaporiser. Vaping itself (mostly in other forms – including the hookah) has a much longer history with the first electronic vaporiser developed by Joseph Robinson in 1927 (US patent: US1775947A).

While potentially beneficial in terms of smoking cessation, vaping devices can deliver significant and, potentially, addicting, nicotine doses [6]. Hajek et al. conducted a large randomised control trial in the UK comparing ECs to nicotine replacement for smoking cessation, both arms also receiving standard-of-care behavioural supports – demonstrating clear superiority of ECs with 1-year abstinence rates of 18 % vs 9.9 %, albeit tempered by an ongoing EC consumption rate of 80 % [7]. However, later "real-world" data cast doubt on this – especially in terms of long-term abstinence; a study by Chen et al. demonstrated no difference in long-term tobacco abstinence (beyond 1 year) compared to conventional nicotine replacement (although EC users were more likely to remain nicotine dependent) [8]. A Cochrane review of ECs for smoking cessation demonstrated an, at best, small benefit over conventional nicotine replacement (primarily with nicotine-containing ECs) with abstinence rates increasing from 6/100 to 10/100 at 6–12 months (moderate quality of evidence) [9]. Notably, ECs lack FDA approval for smoking cessation- though obstetric data do suggest a benefit to vaping over smoking [10]. Thus, ECs for smoking cessation may best be considered a lesser-harm, chronic intervention with a high risk of ongoing use and nicotine addiction rather than a time-limited intervention. Additionally, despite previous successes in reducing smoking rates and nicotine use, present data suggest a worrying rise in alternative nicotine products and vaping among younger people, particularly adolescents, with US data demonstrating a 10 % increase (from 11 %–21 %) in 12th graders from 2017 to 2018 [11–13].

While being marketed as a less harmful and oncogenic option than conventional nicotine products, vaping would appear to be far from benign.. While nicotine is the most commonly vaped substance, it is by no means the only one and recent years have seen a proliferation of other vaped substances – particularly cannabis derivates, which has coincided with recent legalisation/decriminalisation policies. This is of particular note as the recently described entity of vaping-associated lung injury, characterised by acute lung injury or organizing pneumonia, was associated with cannabis/marijuana oil vaping rather than nicotine-based ELs in 71 % of a series by Butt et al. [14]. As a corollary however, the aetiology was unclear and could *not* be ascribed to non-nicotine containing ELs in all cases [14,15].In this review, we aim to discuss the current evidence and ongoing investigations for the oncogenic potential of vaping and vaping products.

1.1. Lung carcinogenesis

As with most forms of cancer, LCa is caused by the progressive acquisition of genetic defects. Along with the skin and alimentary canal, the respiratory system is directly exposed to the outside environment and, thus, particularly sensitive to exogenous toxins and potential carcinogens. Environmental pollution (especially nitrogen oxides and inhaled particulate matter) and radon gas are both highly associated with LCa with approximately 223,000 LCa deaths in 2010 attributed to environmental pollutants and radon gas being (after smoking) the second greatest risk for LCa – while also demonstrating significant synergistic oncogenicity with smoking [16,17].

Inhaled substances may exert their effects directly, such as the carcinogenic components of tobacco smoke: polycyclic aromatic hydrocarbons, amines and benzene or more indirectly via chronic inflammation and metaplasia of the respiratory epithelium in response to particulate matter [18]. In both circumstances, respiratory tissue over a significant area is exposed to the causative agent with a resultant, field effect and the potential for ultimate oncogenesis therein.

Nicotine itself also has potent potential carcinogenic activity by means of its conversion to nitrosamine compounds – especially nitrosamine ketone and nitrosonornicotine [19]. Approximately 80 % of inhaled nicotine is metabolised to cotinine (believed to be non-toxic) and subsequently eliminated via the urine [20,21]. A small percentage (~10 %) of inhaled nicotine appears to undergo endogenous conversion to nitrosamine compounds [22,23]. Nitrosamine carcinogenicity is believed to be a function of increased DNA methylation and, potentially, direct agonist activity on the nicotinic acetylcholine receptor acting to enhance tumour growth, survival and invasion [24].

The direct, mammalian oncogenicity of electronic cigarette smoke (ECS)-delivered nicotine and its nitrosamine products was elegantly demonstrated in a murine model by Tang et al [19]. In this study, a series of FBV/N mice were exposed respectively over a 54-week period to either aerosolised e-liquid; the, apparently, inert organic vehicle or filtered air. ALCa incidence was 22.5 % in the ECS arm *vs.* 5.6 % and 0% in the filtered air and vehicle arms, respectively – with this result achieving significance. An increased rate of urothelial hyperplasia, likely a precursor lesion to invasive carcinoma, was also observed. While previous studies cast doubt on the absorption and significance of ECS-derived nicotine derivatives, Tang et al demonstrate significant levels of the nitrosamine ketone derivative 4-(methylnitrosoamino)-4-(3-pyidyl)-1-butanol in lung tissues [25]. While its systemic absorption is less certain, it appears likely that these derivates achieve sufficient *local* concentrations within the distal bronchioles and alveoli to induce significant DNA damage and adduct formation.

A study by Lee et al demonstrated similar DNA methylation changes from ECS compared to those expected from tobacco smoke (TS) with evidence of DNA adduct formation in murine bronchogenic and other tissues [26]. ECs operate by means of an atomiser vaporising the liquid, which is subsequently inhaled. As the depth of penetration into the respiratory tract is determined by particle size (with smaller particles penetrating more deeply) the submicron aerosols generated by ECs are capable of penetration to the alveolar level. Thus, atomisers of increasing power levels are associated with *larger* particle size and, potentially, more proximal deposition - due to supersaturation and subsequent condensation [27].

Arguably the most harmful component of TS are its aldehyde components (especially acetaldehyde - ethanal, formaldehyde – methanol and acrolein). These organic compounds demonstrate potent inflammatory and toxic activity (also inducing dyslipidaemia, vascular injury and increased platelet reactivity). The resulting inflammatory milieu is associated with macrophage activation and chemotaxis and the resultant generation of reactive oxygen species with secondary immune modulation and the induction of an adverse inflammasome [28,29]. This pathway appears to have multifactorial oncogenicity with inflammation being directly associated with LCa and with the adverse inflammasome/macrophage activation inducing an overall immunosuppressive "cold" environment, hostile to T-Cells – proven to be important in LCa oncogenesis and malignant potential [30].

These aldehydes, especially formaldehyde, are also found in ECS

with levels varying considerably both compared to conventional TS and across different e-liquid formulations, especially flavoured ones. While originally felt to arise from the volatile flavouring compounds, these carbonyl compounds have subsequently been detected (and at comparable or greater levels) in both flavoured and unflavoured ELs [31]. This is understood to arise from the thermal decomposition of propylene glycol, glycerol and other organic EL constituents driven by the heating element – as demonstrated by Ogunwale et al [28]. The degree of production of aldehydes and other carbonyl compounds appears to be directly related to the voltage used and, thus, the temperature of the EC heating coil. Increasing coil temperatures result in a greater potential for pyrolysis, oxidation and thermal decomposition of these organics – with data from Kosmider at al and Sleiman et al suggesting an exponential increase therewith [31–34]. This is discussed further in section 1.3 below.

While some data suggest these very elevated aldehyde levels are primarily produced in so-called "dry-puff" scenarios felt to be uncommon in real-world practice and associated with an adverse user experience, these data are, at best, equivocal [35]. Studies by Conklin et al (in mice) and Samburova et al (in human volunteers) clearly demonstrate detectable and clinically relevant aldehyde levels following exposure to commercially available EL products [34,36]. Given the frequency with which ECs are modified by users (particularly seeking to increase nicotine/EL dose) and resulting increased coil voltages, real-world carbonyl exposure from ECS must be regarded as significant and frequently greater than that of conventional TS.

Aside from its primary oncogenic activity, TS demonstrates both mitogenic and cocarcinogen activity to existing (pre-)malignant lesions. TS also impairs pulmonary clearance of particulates and some toxins by means of a direct suppressive effect on respiratory epithelial cilia [37].

1.2. Vaping devices

Ultimately, all vaping devices exist to deliver an aerosolised EL/VL to the user with devices usually containing 3 central components: a liquid reservoir (which may be refillable or disposable) a heating element (acting to vaporise the EL/VL) and a power source [38,39]. Numerous different designs and styles exist – from the disposable simulacrum cigarette to faux pipe devices to cartridge or tubular-shaped devices; extensive brand-differentiation having been pursued between these. Otherwise, devices may be single-use, disposable (especially in the form of a combined atomiser and pre-filled cartridge – the so-called "cartomizer) or (more frequently) reusable. Within the field of reusable devices, they may accept only proprietary cartridges (thus providing ongoing revenue to the supplier) or they may allow independent refilling (including with non-standard ELs/VLs) – as emphasised by the recent proliferation of vaping shops. While early devices appear to imitate conventional tobacco products, more recent devices appear to resemble other entities – eg the JUUL device apparently imitating a USB drive – and to be products of stylised, modern industrial design, similar to other modern electronics.

An alternative design to vaping devices, relatively unsuccessful until the recent IQOS device, is to heat (arguably without conventional combustion) tobacco directly and deliver the resulting gaseous/vapour products to the user. This has been marketed as a safer device, avoiding the stigmas of both conventional cigarettes and vaping devices – though has not, in fact, been demonstrated to be any safer or to pose any lower risk of LCa or other smoking/vaping-related disease. Indeed, a study by McAlinden et al. demonstrated similar respiratory epithelial toxicity from the IQOS device compared to conventional tobacco smoke – including adverse effects on cellular energetics, EMT and oxidative stress (all associated with potential malignant transformation) [40].

These devices are also open to modification – with an extensive, albeit unofficial, community involved therein. All 3 of the basic constituents of a vaping device are amenable to modification. The liquid reservoir/injection can be modified to increase VL fluid delivery with each activation and/or the fluid itself may be adulterated or mixed.

The heating element (usually a coil) can be modified in a number of ways. Increasing EL delivery usually necessitates increased power output to ensure (near) complete vaporisation, however, increased power outputs may also either burn out the coil or impart adverse flavours – thus some users change coil gauge or metal type. The battery may be replaced (if discharged) or be modified to increase charge delivery – greater VL volumes or different coil characteristics may require altered power delivery. Two studies (one examining YouTube videos, another interviewing a series of volunteers) assessed vaping device modification – modifications to the coil followed by the EL/VL being most common [41,42]. The majority of YouTube videos provided "How To" guides to vaping device modification or home construction – without clearly identifying risks: including fire hazard/explosion (as in the case of improperly managed Lithium batteries).

1.3. Vaping fluids

Fundamentally, the principle of vaping is to deliver aerosolised nicotine and/or other agents to the recipient by means of the respiratory tract with the VL/EL acting as both the nicotine source and the delivery vehicle. While early ELs were largely inert, there was a subsequent shift towards flavoured/perfumed ELs, largely for reasons of improved palatability, as nicotine-alone based ELs proved bitter and unpalatable. This would ultimately culminate in the plethora of flavoured vaping products brought to market in recent years – which increasingly appear marketed as luxury products in and of themselves, rather than as a conventional nicotine replacement. Arguably, they also appear to be directly targeted at low/non-smokers and a younger, adolescent demographic - which will be discussed later [12]. Alarmingly, research has demonstrated that flavoured ELs contained a range of chemical constituents, capable of inducing significant toxicity to the respiratory epithelium [43–46].

The principle of flavoured nicotine products is, however, considerably older with menthol (acting to activate endogenous "cold receptors", transient receptor potential M8 (TRPM8) and to deliver a mint flavour) being added to cigarettes since the early 20th century. As evidenced by the index agent, menthol, these flavourings are far from inert bystanders, demonstrating direct toxic effects on the lung and other tissues and potential oncogenic effects. Of note, specific regulation of these flavoured ELs, beyond the use of agents deemed GRAS (Generally Recognised as Safe – a principle intended for foodstuffs), was largely absent until this year – with the FDA finalising its enforcement policy on ELs and requiring all flavoured ELs (excluding menthol) to be submitted for authorization [47]. Commonly encountered contaminants and additives along with their mechanism of action and associated toxicities are summarised in Fig. 1below.

1.3.1. Menthol

Menthol exerts potential oncogenic effects by two main pathways – modulation of nicotine metabolism and direct oncogenicity/pro-inflammatory effects. Menthol is also associated with overall increased rates of nicotine dependence – by means of both increased tolerance/reduced throat irritation of TS (and, likely, ECS) and direct effects on the endogenous response to nicotine (including by modulating nicotine receptor expression) [48]. This effect appears even more marked in adolescents and young adults (AYAs) [49]. Given the oncogenicity of nicotine and its breakdown products, this results in greater tissue exposure and potential DNA damage.

As demonstrated by Muthumalage et al., menthol also induces a local pro-inflammatory microenvironment with activation of monocytes and pro-inflammatory cytokines (IL-6, IL-8, PGE_2), increased superoxide dismutase (SOD) expression and increased reactive oxygen species. In this study, cells from various lines (including: U937, 16-HBE and BEAS-2B) were exposed to the aerosolised products of EL combustion from a variety of EL flavours from JUUL device via a Scireq Inexpose e-cig

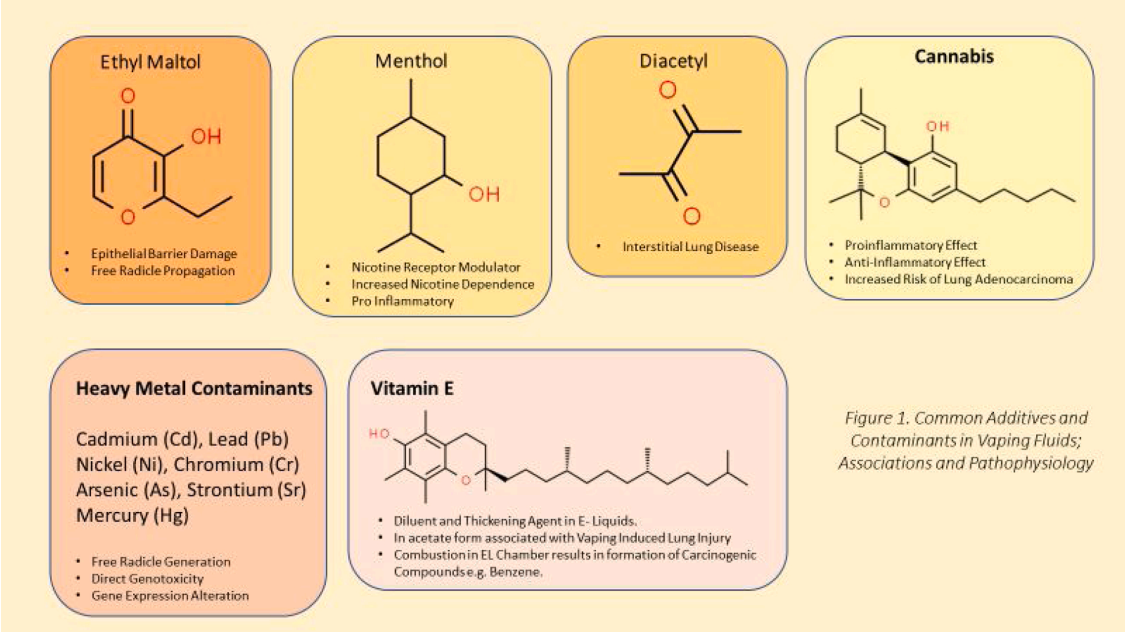

Figure 1. Common Additives and Contaminants in Vaping Fluids; Associations and Pathophysiology

Fig. 1. Summary of Common Additives and Contaminants in Vaping Fluids; Associations and Pathophysiology. A brief summary and review of the molecular structure and functional groups of commonly encountered VL additives and contaminants along with their associated toxicities and mechanisms thereof.

exposure system and Enzyscreen chamber. Thus, the *in vitro* biological effect of the ELs (including the menthol component) were assessed, including both the combustion products and any unburnt menthol. This milieu demonstrated direct DNA damage (by Comet assay) and, thus, probable oncogenicity [50]. Another study by Zahedi et al. demonstrated increased epithelial-mesenchymal transition (EMT) in A549 CCL-185 lung cancer cells, with resultant increased invasive/metastatic potential, on exposure to various flavoured ELs – including menthol based [51].

Furthermore, Nair et al. demonstrated that the pro-inflammatory effects of menthol were mediated directly via TRPM8, resulting in calcium influx in a BEAS-2B cell-line model [52]. Interestingly, altered intracellular calcium through TRPM8 has been previously shown to induce a neoplastic phenotype in LCa [53,54].

1.3.2. Ethyl maltol

Ethyl maltol is used to impart a sweet, caramel flavour and is present in many vanilla, candy and fruit-flavoured ELs. Bitzer et al. demonstrate significant, *in vitro*, free radical generation in vaped ethyl maltol. It has also been shown to interact with iron and copper (frequently present in the heating element and/or as contaminants) to form hydroxypyranone complexes – resulting in further radical generation [55].

It has also been demonstrated to induce both an inflammatory response and alterations in local immune function and to compromise epithelial barrier function and integrity – with promotion of further proinflammatory effects and with increased systemic exposure of inhaled substances [56,57]. Given the demonstrable oncogenicity, both as single agents and synergistically, of free radicals, this strongly suggests the oncogenicity of ethyl maltol.

1.3.3. Diacetyl

Diacetyl represents perhaps the index example of a flavour compound demonstrating profound lung toxicity. Used to provide a buttery flavour, it (along with related diketones such as acetoin) is a key ingredient in synthetic butter flavoured foods – especially butter popcorn. It originally came to prominence following the association of butter flavoured popcorn with lung disease, primarily bronchiolitis obliterans, ultimately found to be a toxic effect of diacetyl: the so-called "popcorn lung" [58].

Diacetyl and other diketones are similarly utilised in several common ELs to impart a buttery flavour, a study by Allen et al. demonstrating diacetyl (along with the related compounds pentanedione and acetoin) were present in 47/51 sampled ELs. [46]. Although not itself a proven human carcinogen, the interstitial lung diseases with which diacetyl is associated do, particularly in the more advanced stages, have a demonstrable potential for malignant transformation. Such malignancies have an overall worse prognosis compared to non-interstitial lung disease related LCa - likely relating, at least in part, to underlying lung damage and impaired spirometric parameters/diffusing capacity [59].

On a more general level, many flavouring compounds (as volatile organic compounds) have the potential to undergo pyrolysis/thermal decomposition yielding aldehydes and ketones. Additionally, particularly at the temperatures present in vaping device heating elements (and, possibly, catalysed by the metal component of the element itself), both polyethylene glycol and glycerol – the primary solvents/chemical vehicles utilised in ELs – can also undergo pyrolysis/thermal decomposition, yielding similar end-products [60]. The resulting aldehydes, ketones and other volatile organics are far from benign with several probable or definite human carcinogens, including acrolein and formaldehyde, being demonstrated in EC discharges.

Such volatile organic compounds, especially aldehydes, have been demonstrated by Wang et al. (both in vitro and in animal models) to induce both DNA damage (generally by forming DNA adducts) and to impair DNA repair mechanisms (experimentally reducing activity of XPC and OGG1/2 DNA repair proteins) [61].

Interestingly, Wang et al. also demonstrate that these aldehydes are likely of greater significance than the nicotine derivatives (sections 3.2, 3.4) nitrosamine ketone and nitrosonornicotine as the oncogenicity of these nicotine derivatives requires activation via the CYP pathway, itself inhibited by thesealdehydes. In addition, the range of compounds produced from various flavourings have been shown to contribute to chronic inflammation, ER stress and abnormal growth through altered cytosolic calcium, all of which have been linked to LCa pathogenesis

[44].

The significance of aldehydes in LCa oncogenesis is further supported by the increased rates of LCa seen in Japanese smokers with hereditary aldehyde dehydrogenase 2 deficiency – resulting in functionally greater aldehyde exposure. In this study, odds ratio was 23.2 for LCa development in homozygous deficient cases [62].

1.4. Cannabis and other cannabinoids

We have focussed so far on conventional nicotine based ELs. While the majority of ELs are nicotine-based, there has been a significant rise in the consumption of cannabis and cannabinoid-based ELs, particularly in the younger demographic – with rates of up to 16.6 % in Canada, 13.8 % in the US and 9.0 % in the UK [63,64]. The oncogenic potential of cannabis and its derivatives remains somewhat equivocal – as does its ultimate impact on lung function.

While originally believed to be similarly toxic to lung tissue as TS, conventional cannabis smoking appears to be less associated with parenchymal lung diseases – though it is associated with bullous lung disease [65,66]. The association between cannabis smoking and LCa is similarly equivocal with various series yielding conflicting data – albeit of limited quality (primarily case-control and population studies) and relatively small numbers [67,68].

Present data would suggest that, while cannabis smoke does have toxic and pro-inflammatory effects and is capable of causing pulmonary irritation, it also has direct anti-inflammatory effects which would appear to modulate and, at least in part, mitigate the toxic effects thereof. A metanalysis of cannabis smoking and LCa risk by Zhang et al failed to demonstrate an increased risk of LCa with cannabis consumption. [68]. Data for LCa risk and cannabis containing ELs is even more limited. However, regulation of cannabis ELs is more limited than that of conventional nicotine ELs – presently under review by the FDA – raising significant concerns regarding the purity and variability across products of cannabis ELs.

Furthermore, as with flavouring compounds, the constituents of cannabis ELs are prone to undergo pyrolysis and thermal decomposition yielding various volatile and potentially toxic organic species. This is further compounded by the relatively poor control and high thermal variability in most mainstream cannabis ECs – excepting such higher-end devices as the Volcano (retailing for $470-$700 on average) [69].

A study by Thomas el at, while specifically exploring cannabinoid activity and focussing on synthetic cannabinoids, demonstrated significant and quite unpredictable pyrolytic organic reactions under conditions present in ECs [70]. Potentially oncogenic products were not measured, however this would, in principle at least, suggest a mechanism for the generation of such products, with sufficient basic science rationale for concern at least.

Thus, while a definitive association between cannabis vaping and LCa is presently lacking, there are considerable grounds for concern and a need for further research and scholarship in this area – especially as cannabis EC use is only likely to continue to grow.

1.5. Adulterants/contaminants

Aside from the active agent (usually nicotine or a cannabinoid), vehicle/s and flavourings, ELs frequently contain either intentionally or resulting from contamination, a number of other compounds. Many of these are, again, far from inert. A series of analyses and reviews (including but not limited to: Etter et al., 2013; Famale et al., 2015; Goniewicz et al., 2015) have demonstrated very marked variance between the reported and measured constituents of a series of analysed ELs. These include: nicotine, albeit at trace levels, present in “nicotine free” products, measured nicotine levels varying >20 % from stated levels and a series of contaminants including: Cis-N-oxide, trans-N-oxide and myosmine (at levels which, although exceeding regulations, are likely not toxic) [71–73].

1.5.1. Vitamin E

Vitamin E, especially as the acetate, is an oily substance at room temperature which has been used as both a diluent and thickening agent in several ELs – particularly those containing cannabis derivatives [74]. Although initially felt to be benign and relatively biologically inert, Vitamin E acetate has, in fact, been implicated in the recent series of electronic vaping-associated lung injury cases recently described [75,76]. This disorder, characterised by a generalised lung injury with ground glass infiltrates (diffuse alveolar damage) and, in some series, a lipoid pneumonia with foamy macrophages has been associated with Vitamin E – particularly in association with cannabis EL products [74,77,78].

Aside from its defined acute pulmonary toxicity, Vitamin E and its derivatives have also been demonstrated to undergo pyrolysis and other organic reactions in the conditions present at the heating element of an EC – which, in many cases, behaves like a crude laboratory pyrolysis device. Wu et al. demonstrated that the thermal decomposition of Vitamin E yields highly toxic and irritant ketene gas (via elimination of the aryl acetate group) along with a number of other toxic, reactive species with demonstrable carcinogenic activity, including benzene and various alkenes [79]. They also observe, with excellent rationale, that these reactions are by no means easily predictable with many potentially unexpected end-products – particularly in the setting of any other contaminants/organic compounds and variable quality and contents of the EL.

1.5.2. Heavy metals

Heavy metals such as cadmium, arsenic, mercury and nickel are proven oncogenic agents – although the precise mechanism/s of oncogenesis remain to be fully elucidated. Postulated mechanisms include: oxidative stress by means of free-radical generation (at the level of the electron transport chain in the mitochondrion), direct genotoxicity by metals/metal ions and alterations in stem cell function/gene expression [80,81]. Aside from general and vitro oncogenicity, heavy metal particulates have also been specifically associated with LCa – as in an Italian study by Buonanno et al. demonstrated [82].

This has raised significant concerns for their potential oncogenic role in various ELs – both due to contamination during/after production and due to endogenous mobilisation from the EC, primarily from the heating element. This heating element is usually made from a metal/metal alloy, particularly copper for its electrical and thermal properties, and frequently electroplated/coated with another less-reactive substance. Other metals/alloys are also frequently present - including as solder at joints. Given the temperatures and conditions present, especially with the synchronous generation of volatile organic vapours (and likely novel, reactive organics), there is significant potential for the mobilisation of heavy metal ions from this reservoir. Notably, a number of the organic compounds present in ECS (particularly aldehydes and ketones) either react directly or form complexes with metal ions.

A study by Williams et al. demonstrated both macroscopic evidence of corrosion of the element and the generation of aerosolised microparticles in the 1−20 μm range– with resultant in vivo exposure of the user to this milieu [83]. Analysis of the microparticles demonstrated a multitude of probable toxins including the known carcinogens: lead, chromium, strontium and nickel. They also demonstrated a series of compounds known to cause both fibrotic and inflammatory lung diseases (including severe pneumoconioses) including: boron, silicon (potential for silicosis), iron, barium, aluminium and inorganic tin (potential for stannosis). This was also explored by Hess et al. with similar findings [84].

Notably, both of these studies assessed combined atomiser/fluid cartridges – the so-called “cartomizer” - a disposable device. Reusable devices usually include a separate, replaceable, heating coil and EL tank with coils replacement based on usage. Olmedo et al. separately assessed the metal concentrations in the EL (prior to exposure to the vaping device), in the EL tank and in the aerosol produced by the device when in

use in a population of EC device users recruited for another study using their personal devices [85]. This study clearly demonstrated leaching of heavy metals (including: Al, Cr, Mn, Ni, Pb, Sn, Zn) from the device, likely both tank and heating coil, into the EL with minimal, if any, detectable metal levels in the baseline EL, markedly increasing when measured in the tank and aerosol. Metal levels were greatest in the tank (excepting for Zn, which was greatest in the aerosol) but remained significantly elevated in the aerosol also. Frequency of coil-change was associated with greater metal concentration within the aerosol but not the tank – again supporting both tank and coil as contamination sources. This would suggest a *potential* advantage to single-use devices due to potentially reduced heavy metal exposure – particularly from the tank present in multi-use devices.

This would strongly suggest a potential oncogenic risk from heavy metal contamination in ECS – likely both from the devices themselves and the ELs. Furthermore, this is only likely to become a greater issue and to further increase toxic metal exposure with prolonged EC device use (due to increasing exposure and reaction of the metal components). Any of the, not infrequently undertaken, modifications made to these devices – particularly increasing power output and/or EL dose – are only likely to exacerbate this risk.

1.6. Molecular mechanisms of oncogenesis

A number of different molecular mechanisms have been suggested for the oncogenic effect of ECs/ELs – relating to both the direct chemical constituents thereof and potential daughter products of combustion and pyrolysis [86]. Specific evidence exists for EMT, redox stress/mitochondrial toxicity and for DNA breaks/fragmentation. The most common mechanisms for cellular and genetic toxicity and their association with malignant transformation are summarised in Fig. 3 (7.3) below.

1.6.1. Epithelial-mesenchymal transition

EMT is one of the fundamental characteristics of a cancer cell, as described by Weinberg et al., in which epithelial cells acquire mesenchymal characteristics including motility and, subsequently, the capacity for invasion and metastasis. EMT is a known factor in LCa oncogenesis and definitively associated with TS – by means of various genes including those coding for transcription factors (FOXC1, FOXC2, FOXQ1, FOXM1 etc), zinc-finger proteins (SNAIL1, SLUG, ZEB1 etc), cellular adhesion molecules (E-cadherin, N-cadherin) and α-smooth muscle actin. A series of TS constituents have been demonstrated to induce EMT via these pathways experimentally – including direct cellular effects by nicotine itself (as discussed above) and gene expression modulation by means of methylation [87–89]. This pathway is important in the pathophysiologies of chronic obstructive pulmonary disease (COPD) and pulmonary fibrosis – with their associated increased risks of LCa [90]. EMT has also been demonstrated in vitro by Zahedi et al. in A549 lung cancer cells by ECs (containing both menthol and tobacco ELs) with acquisition of fibroblast-like morphology, loss of cellular adhesion molecules and increased motility [51]. The mechanisms for EMT along with changes in phenotype and acquisition of invasive potential are summarised in Fig. 2 below. Given the proven and fundamental association between EMT and invasive cancer and its importance to LCa oncogenesis and tobacco-induced cancers, this would appear similarly relevant to EC/EL oncogenesis.

1.6.2. Redox stress/mitochondrial toxicity

Reactive oxygen species and resulting free-radical generation are associated with oncogenesis and a postulated mechanism of heavy-metal toxicity and oncogenicity (section 6.1.2) [81]. A study by Lerner et al. expands on this demonstrating unequivocal sensitivity of mitochondria to EC aerosols and copper nanoparticle-containing EC aerosols in cultured human lung fibroblasts. This resulted in deregulated cellular energetics (by means of mitochondrial electron transport chain disruption), inflammation and genotoxicity [91]. Current data suggest a critical role for mitochondria and mitochondrial subversion in cancer (dating back to Warburg's observations on cellular energetics and glucose metabolism in cancer cells in the 1920s) – including altered mitochondrial metabolism/reactive oxygen species generation and mitochondrial genome disruption [92–94]. While an area of ongoing investigation, redox stress/mitochondrial toxicity is likely of considerable importance to cancer oncogenesis in general and LCa oncogenesis in particular with ECS/ELs having demonstrable effects thereon.

1.6.3. DNA breaks/fragmentation

Cancer is inherently a genetic disease, associated with the

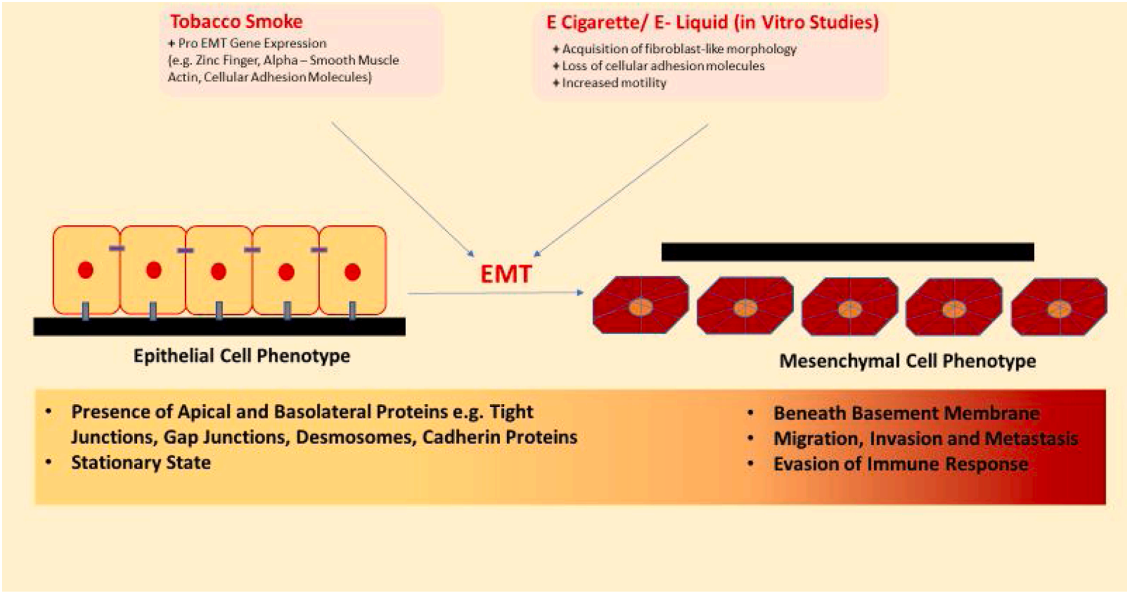

Fig. 2. Associations between Tobacco Smoke, E-Cigarettes and Epithelial - Mesenchymal Transition. An overview of the mechanism for TS- and ECS/EL-induced EMT including changes in cellular and tissue phenotype with acquisition of motility and invasive/metastatic potential.

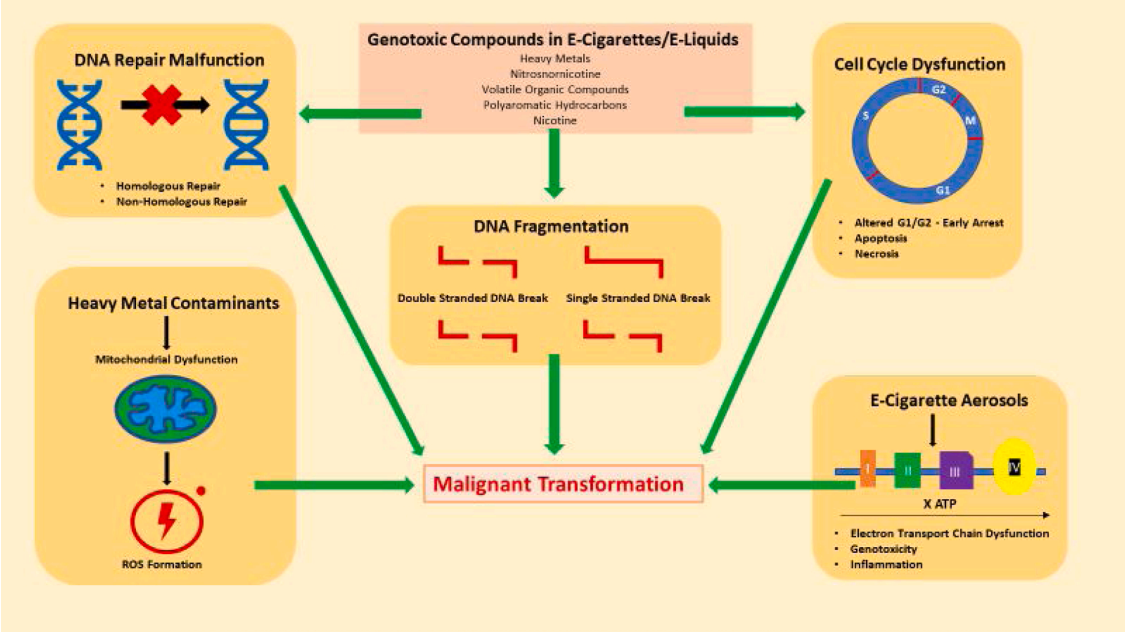

Fig. 3. Impact of EC's/EL's on DNA Structure, Mitochondrial Function, DNA Repair and the Cell Cycle. A brief overview of the most common mechanisms of cellular and genetic toxicity associated with ECS/ELs and their association with malignant transformation.

progressive acquisition of genetic defects with cancers themselves exhibiting genomic instability and clonal evolution. Thus, any mechanism capable of inducing DNA damage or breaks (particularly double-stranded breaks) is capable of oncogenesis. As discussed above, ECS contains various genotoxic compounds (both primarily and as daughter products of combustion/pyrolysis) capable of inducing both single- and double-stranded DNA breaks and DNA fragmentation/mutation, including: heavy metals, nitrosnornicotine, various volatile organics and polyaromatic hydrocarbons and reactive oxygen species - which are also capable of inducing direct DNA damage. The most common mechanisms for cellular and genetic toxicity and their association with malignant transformation are summarised in Fig. 3 above. As demonstrated by Yu et al., ECS/ELs demonstrate clear genotoxicity, inducing DNA damage (both single- and double-stranded breaks) in vitro, utilising HaCat, HN30 and UMSCC10B cell lines and the neutral comet assay. They demonstrate significantly increased rates of DNA breaks over control with both nicotine-containing and nicotine-free ECs (though genotoxicity was greater with nicotine-containing ECs); perhaps tellingly, genotoxicity of nicotine-containing ECs was approximately equivalent to that of conventional tobacco products. This was also associated with an altered cell cycle (early G1 and G2 arrest) and increased apoptosis and necrosis with resultant cell death by both pathways [95].

The generation of double-stranded DNA breaks demonstrated here is especially indicative of ultimate carcinogenic potential as this form of DNA damage is predominantly repaired by the non-homologous end joining pathway. This pathway is particularly error-prone and associated with the progressive acquisition of deletions and mutations [96]. Homologous repair (a higher-fidelity repair pathway, already less active in this situation) is further suppressed as it is most active in S-phase – thus sensitive to ECS effects on cell cycle. Consequently, prolonged use of ECs would appear to lead to progressive rounds of DNA damage followed by dysfunctional repair and the progressive acquisition of mutations and other genomic aberrations, ultimately culminating in definitive malignant transformation. Given the demonstrable genotoxicity of various ECS constituents, this pathway must be considered relevant to their oncogenic potential.

1.7. Demographics of EC use

While the current generation of ECs were originally introduced as a form of nicotine replacement therapy, they have evolved into an object of hedonic consumption in and of themselves. Surveys demonstrate that most consumers believe them to be healthier (88.2 % believing them less harmful and 11 % believing them absolutely safe compared to TS in a >19,000 responder survey by Farsalinos et al) and to both improve overall physical health. In many cases, they are also believed to lower total nicotine exposure [97–99]. Of note, many respondents praised ECS cloud formation, voltage adjustability and the aesthetic of the device – further supporting the role of the EC as a luxury device in and of itself.

In direct opposition to their potential role in smoking cessation is the potential for ECs to act as an entry point into nicotine use and subsequent addiction, essentially a gateway drug. Frequent use normalises and leads to subsequent smoking in ex-smokers, those trying to quit, and those who never previously smoked leading to increased tobacco or other recreational drug consumption. Indeed, there is both theoretical rationale and neurobiochemical evidence for this. A review by Sven Schneider et al., synthesising existing evidence for ECs as a gateway to conventional tobacco demonstrates ECs initially acting to normalise nicotine consumption and alleviate concerns regarding the consumption thereof with the resultant familiarity later potentially culminating in the transition to tobacco consumption, especially in younger demographics – the "catalyst" model [100]. The underlying neurobiochemical mechanism for this was explored by Kandel et al. with nicotine inducing global acetylation in the striatum (via histone deacetylase inhibition) with secondary alterations in gene expression and inhibition of long-term potentiation in the nucleus accumbens.Ultimately this results in disinhibition of dopaminergic neurones in the ventral tegmental area and increased dopamine secretion – the same mechanisms seen with conventional tobacco products. In this study, this significantly increased the physiologic effect and, thus, likely the potential for addiction, of cocaine [101].

The rising rate of EC use in younger demographics is of particular concern – as noted by the Monitoring the Future study, Johnston et al, 2019(102). This large survey demonstrated a very marked rise in

nicotine-based EC use amongst adolescents with increases of 3.4 %, 8.9 % and 10.9 % in 8th, 10th and 12th US grades yielding prevalence rates of 11 %, 25 % and 30 % respectively; similar (or slightly greater) increases in marijuana-vaping yielding prevalence rates of 4.4 %, 12.4 % and 13.1 % respectively. Prevalence rates of *any* vaped substance (including flavouring alone) were 18 %, 32 % and 37 % for this demographic.

Of perhaps more concern is the perceived risk of EC and EL products with only 14 %–15 % of adolescents perceiving any significant risk to the use of EC/EL products in 2014. Risk perception did increase with time - reaching 22 %–23 % (8th and 10th grade) and 18 % (12th grade) in 2018 – though it remained amongst the lowest perceived risk levels for all drugs, including alcohol. Additionally, perceived risk of ECs/ELs compared to conventional tobacco products actually *reduced* with increasing age [102].

This rise in EC/EL use in the younger demographic appears to coincide with the aggressive and, arguably, directly youth/adolescent-targeted advertising campaigns of a number of EC/EL manufacturers, particularly JUUL labs – culminating in an FDA warning regarding their advertising practices [103]. Notably, the fundamentals of these advertising campaigns bore significant (and, arguably, worrying) similarity to the advertising campaigns and sponsorships of conventional cigarette and tobacco corporations from earlier decades – with their known impact on tobacco use amongst teens and adolescents [104].

Given the long lag times between carcinogen exposure and the ultimate development of a malignancy (traditionally stated as 20 pack/years for a significantly increased risk of LCa with conventional TS exposure – though this may be an overestimate, as per more recent data), youth consumption of ELs/use of ECs is of particular concern. Additionally, even nicotine-/marijuana-free, flavouring only, vaping would appear far from benign – based on the potential toxicities of common flavourings and potentially toxic organic combustion products of ELs. Ultimately, cancer risk in the younger demographics has a considerably greater population and economic impact as potential LCa cases are likely to be in their prime earning years and/or raising families.

2. Discussion

At present we lack unequivocal, epidemiological evidence of an association between EC use (either nicotine-based or other) and LCa. There exists, however, extensive data and excellent scientific rationale to suggest a significantly increased risk of LCa therewith. Many substances identified within ECS are either unequivocal or probable human carcinogens – including nicotine (and its derivatives) itself. Furthermore, the lack of definitive epidemiologic data at present is hardly surprising given the lag periods between oncogenic exposure and ultimate malignant transformation, along with the only quite recent marked expansion in EC/EL use – we simply have not yet had time to observe the true impact thereof. Additionally, the numbers of dual users of both traditional tobacco products and ECs are increasing, thus the risk posed by this dual use requires further investigation.

In the short term further clinical and basic science research might help identify key toxicological and biological effects of ECS on the respiratory epithelium and potentially identify alterations that are associated with the development of LCa. Current studies lackappropriate patient samples and respiratory models and utilise inconsistent exposure methods. As a result, it is difficult to draw clear conclusions across studies; going forward these areas need to be improved. Clinical research needs to ensure a clear separation in patient populations of those who are termed “dual users” having previously smoked and those who are “never users”, in order to clearly discern the effects of ECS [105]. In particular, it is vitally important we understand the impact of ECS on never users, however obtaining clinical samples from this population who currently appear relatively healthy may prove difficult.

Laboratory models can potentially bridge this gap, however a large proportion of research conducted to date use cell lines grow as 2D submerged cultures, which do not replicate human biology nor aerosol exposure [105]. The use of human samples grown as a 3D pseudostratified respiratory epithelium at air liquid interface overcomes this issue by more closely mimicking in vivo conditions [105]. There also needs to be more consistency around the methods and protocols employed to generate vapours from ECS, ensuring the delivery and exposure is similar to human ECS inhalation [106]. To date there have been a wide variety of methods employed resulting in the varying concentrations and exposure time of ECS, in addition to the numerous ELs producing varying toxic compounds further adding to the complexity and inconsistency [43–45,107].

While there may be a role for ECs/ELs as a means to facilitate smoking cessation/reduction on the grounds of a risk-reductive strategy (though further evidence is required), caution and prudence must be applied [108]. ECs are increasingly being used by previous non-smokers, in particular adolescents (section 81.7), and appear to be acting as a gateway to nicotine addiction. Clearly the risk-benefit analysis is firmly against their use in this setting. Fortunately, this would appear to have been recognised with recent FDA actions to address and reverse marketing strategies which would appear to be directed towards a younger demographic – follow up of both youth and adolescent opinion and utilisation of ECs/ELs over time will be most interesting from this perspective.

Given that the *raison d’être* of ECs/ELs (at least according to the originator of the present generations thereof) is smoking cessation, this would suggest limiting their use to those who stand to benefit from this. A rational argument can thus be made for controlling the sale of and limiting/banning direct advertising of ECs.

3. Conclusion

While definitive data are presently lacking, the authors feel the currently available data *do* strongly suggest an association between ECs/ELs and an increased risk of LCa. Given the lag period between carcinogen exposure and invasive malignancy, this may not become evident for a significant further time period. Furthermore, while LCa risk is likely greatest with nicotine based ELs, there is clear evidence of carcinogenic potential within the “nicotine free” ELs also – particularly in the form of flavourings, additives and contaminants (especially heavy metals derived from the device). Thus, further research in this area is necessary – with a focus on longer-term follow up. To this end, we, the authors, would strongly advocate additional resource allocation (at both European and global levels) towards investigation of ECS/EL-associated LCa (and other lung disease) risk. Additionally, we would advocate expansion of existing registries and biobanks to include EC/EL exposure data and/or the creation of EC/EL-specific registries/biobanks.

We also feel current data demonstrate, at best, very limited evidence for ECs in the facilitation of smoking cessation. On the other hand, there is ample, clear data to support their role as a gateway into nicotine addiction – with no evidence for vaped nicotine being any less addictive or harmful than that derived from conventional TS. Given their significant and increasing consumption by the AYA demographic, we strongly advocate both education regarding the risks of ECs/ELs and controls on their sales and marketing – with a focus on the AYA cohort.

There is an urgent need for greater public awareness regarding the, at present, unknown long-term effects of vaping on not only LCa risk but lung health in general. The current portrayal of vaping devices as a “healthier” alternative with tag lines such as “safer than smoking” and as an aid towards smoking cessation are, at best, lacking evidence and, at worst, a gross misrepresentation of current data – a comparison to filter tips as “the safer cigarette” would seem apt. In light of this, policy makers and public health officials must tackle the misinformation around these issues – in the absence of randomised control trial data to support these claims. Thus, we feel that future smoking cessation campaigns should encompass education on vaping, with clear messaging regarding their health risks, strong potential for nicotine addiction (especially given the increased nicotine delivery by ECs) and association

with later tobacco consumption. In light of the current paucity of risk/safety data, we would also advocate consideration for vaping bans in enclosed public areas, given the potential dangers of "passive vaping" – akin to the smoking bans successfully rolled out in various jurisdictions. Globally, tighter regulation is required - especially in terms of appropriate labelling of vaping products and AYA cohort-direct advertising.

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Transparency document

The Transparency document associated with this article can be found in the online version.

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