Abstract: 2-Amino-9H-pyrido[2,3-b]indole: a potential colorectal carcinogen formed in tobacco
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Epidemiologic studies conducted over the past two decades have consistently shown that tobacco smoking is a risk factor for cancers of the gastrointestinal tract (GI cancers) (1-3). There is also mounting evidence that tobacco smoke is an independent risk factor for hepatocellular carcinoma, the predominant form of human liver cancer (4-6). However, the causal agents of these cancers in tobacco smoke remain to be determined. 2-Amino-9*H*-pyrido[4,3-*b*]indole (AαC), an heterocyclic aromatic amine (HAA) pyrolysis product of protein, is present in main stream tobacco smoke at far greater quantities than other carcinogenic HAAs, aromatic amines, such as 4-aminobiphenyl (4-ABP) and 2-naphthylamine (2-NA), which are implicated in the pathogenesis of bladder cancer (7,8), or polycyclic aromatic hydrocarbons, including benzo[a]pyrene (B[a]P), a potential human lung carcinogen (9). The levels of AαC are comparable to those of tobacco-specific nitrosamine 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), which is believed to be a human lung carcinogen (9,10). AαC is a liver carcinogen in mice, and both a potent *lacI* transgene colon mutagen and an inducer of aberrant crypt foci, early biomarkers of neoplasms, in the colon of mice (11-13). Our recent findings show that AαC undergoes bioactivation to form DNA adducts at very high levels in human hepatocytes (14). Hepatocellular carcinoma is a rapidly fatal human cancer, with median time between first diagnosis and death being approximately 3 months (15). Therefore, new knowledge on causal agents responsible for this fatal human cancer, which has been increasing in incidence in the US during the past 3 decades (15,16), carries significant public health implications.

The *long-term goal* of our parent Grant 5R01CA134700-03 (PI: R. Turesky, Project Title: 2-Amino-9*H*-pyrido[2,3-*b*]indole: a potential colorectal carcinogen, funded in 2010, was to determine if AαC in tobacco smoke is causally related to GI tract cancer development in human smokers, and to elucidate the mechanistic pathways underlying this exposure-cancer relationship. There was no non-human, in vivo information concerning the carcinogenicity of AαC in the colon. Therefore, the specific aims of the parent grant were ***1***) to independently confirm our earlier observation (17) of a statistically significant, and dose-dependent association between cigarette smoking and AαC exposure among Chinese, on a population-based sample of non-Hispanic White Americans, ***2***) to establish an animal model for AαC as a colorectal carcinogen, and ***3***) to elucidate the metabolism pathways of AαC, especially as they relate to DNA adduct formation in colorectal tissue, to support the biochemical plausibility of a role for AαC in human GI tract cancers. These multi-step studies are essential for understanding the biochemical toxicology of AαC. However, if we are to link a role for AαC in human digestive tract cancers, stable, long-term biomarkers of AαC must be developed and implemented in population-based, molecular epidemiology studies. The *objective* of this grant revision is to examine the reaction products of the carcinogenic metabolites of AαC with serum albumin and hemoglobin, in order to achieve the eventual goal of developing AαC adducts of these blood proteins as potential human biomarkers. We have had a productive, decade-long collaboration with Dr Mimi Yu, co-founder of two population-based, large-scale prospective cohorts with baseline blood and urine specimens (18-21), which will be used to definitively test the hypothesis that AαC is a causal agent of tobacco related GI cancers, especially the invariably fatal hepatocellular carcinoma in humans

In summary, the current proposal directly responds to the PAR-12-010 FOA's stated intent, that "Projects resulting from this FOA are expected to serve the FDA by generating relevant findings and data

needed to inform the regulation of the manufacture, distribution, and marketing of tobacco products to protect public health." More specifically, our proposal explicitly addresses the first two bullet points under subheading #3 (Reducing Toxicity of Tobacco Products and Smoke) of this FOA's Specific Research Objectives and Scope: ***1***) What methods and measures best assess biologically relevant changes in harmful and potentially harmful constituents in tobacco products and smoke in both animal models and humans?, and ***2***) Are there in vitro or in vivo assays that can be used to compare overall toxicity between two different tobacco products; with special attention to cardiotoxicity, respiratory toxicity, carcinogenicity and developmental/reproductive toxicity?