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Black raspberries in cancer clinical trials: Past, present and future

Laura A. Kresty[a,*], Susan R. Mallery[b] and Gary D. Stoner[a]

[a]*Department of Medicine, Division of Hematology and Oncology, Medical College of Wisconsin, Milwaukee, WI, USA*

[b]*Division of Oral and Maxillofacial Pathology and Radiology, College of Dentistry, The Ohio State University, Columbus, OH, USA*

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Abstract.

BACKGROUND: Black raspberries (BRB) inhibit a broad range of cancers in preclinical models, including *in vivo* models of oral, esophageal, colon, breast and skin cancer. Promising preclinical results have led to clinical evaluations in cancer patients or patients at increased risk for cancer development.

OBJECTIVE: To summarize clinical investigations targeting cancer or precancerous lesions with BRB and discuss future directions.

METHODS: A thorough literature search was conducted through December 1, 2015 to identify all published studies evaluating BRB in cancer focused clinical trials.

RESULTS: Research investigating BRB in clinical settings report positive effects on preneoplastic lesions or cancers of the oral cavity, esophagus and colon. BRB treatment resulted in: histologic regression of oral intraepithelial neoplasia associated with improved histologic grade and significantly reduced loss of heterozygosity at tumor suppressor gene loci, modulated genes linked to RNA processing and growth factor recycling; in the colon, BRB inhibited FAP-associated polyp progression, demethylated tumor suppressor genes and improved plasma cytokine profiles; in Barrett's patients, BRB consumption increased tissue levels of GST-pi and decreased 8-isoprostane, a marker of lipid peroxidation/oxidative stress.

CONCLUSIONS: The precise dose, duration and optimum mode of BRB delivery for cancer inhibition remains to be fully elucidated. Common themes across studies support that BRB are anti-proliferative, anti- inflammatory, reduce oxidative stress and restore tumor suppressive activity. Future directions are included in the conclusions section.

Keywords: Cancer prevention, black raspberry, oral cavity, esophagus, colon, human clinical trial

1. Introduction

Over the last decade a numerous lines of evidence have converged to support clinical investigations utilizing black raspberries (BRB) as inhibitors of cancer or premalignancy in high risk human cohorts. First, mounting epidemiological evidence shows increased consumption of plant based diets is associated with decreased cancer risk, particularly cancers of the aerodigestive tract [1–4]. Second, strong preclinical results in animal models report that BRB inhibit cancers of the oral cavity, esophagus, colon, breast and skin through targeting processes

*Corresponding author: Laura A. Kresty, Medical College of Wisconsin, Division of Hematology & Oncology, Department of Medicine, TBRC #3910, 8701 Watertown Plank Road, Milwaukee, WI 53226, USA. Tel.: +1 414 955 2673; E-mail: Lkresty@mcw.edu.

of proliferation, inflammation, angiogenesis and apoptosis [5–14]. Third, from a composition stand point black raspberries are rich in vitamins, minerals, fiber, anthocyanins, total phenolics and other bioactive components with cancer inhibitory capacity as previously reviewed [15]. Lastly, initial research reported that maximum concentrations of the major anthocyanins and ellagic acid occurred at 1 to 2 hours in the plasma and between 30 minutes and 4 hours in the urine following BRB consumption with less than 1% overall uptake of the parent or precursor anthocyanins raising concerns regarding bioavailability; however, more recent research shows bioavailability of anthocyanins is comparable to other flavonoid subclasses with significant uptake of diverse metabolites with half-lives ranging from 2 to 96 hours [16]. In turn, the metabolites reportedly modify cellular adhesion and inflammatory signaling cascades, both important cancer associated processes [16].

2. Materials and methods

A thorough bibliographic search was conducted utilizing PubMed through December 1, 2015 to identify all published clinical interventions utilizing black raspberries to target cancers or premalignant lesions. Keywords searches included black raspberry, black raspberries, cancer, premalignant, premalignancy and clinical trials in combination to ensure all completed published investigations were identified. Clinical research with BRB has been completed targeting cancer or premalignancy of the oral cavity, esophagus and colon [17–25] and will be reviewed herein. Individual clinical trial details are reported or referenced in the section summarizing each research investigation.

3. Results

3.1. Pilot study of BRB gel in patients with oral premalignancy

An estimated 60% of cancers of the mouth, pharynx and larynx may be prevented by eliminating tobacco use, reducing alcohol intake and increasing consumption of fruits and non-starchy vegetables [4, 26, 27]. Among never smokers, high fruit consumption is strongly protective against head and neck cancers. Mallery and colleagues designed two studies which effectively build upon epidemiological findings and extend positive preclinical research into clinical trials in at risk patients with oral premalignancy [17, 18]. A 6 week trial was conducted utilizing a 10% BRB gel to target oral premalignancy, ranging from hyperkeratosis with atypia to severe dysplasia [17]. Details of the gel preparation have been previously documented [17–19, 28, 29]. In brief, the gel contained 10% freeze dried black raspberry powder and was pH 3.5 to stabilize the more biologically active flavylium cation of the anthocyanin molecules [17]. Patients applied the gel 4 × daily delivering 0.5 g of BRB in each application. The study details and inclusion criteria which included patients 18 years of age or older with microscopically confirmed premalignant oral epithelial changes and no recent use of tobacco products have been previously published in two reports [17, 18]. The first and second investigation evaluated 17 and 20 patients, respectively with oral premalignancy and 10 patients with histologically normal oral epithelium. The study approach included removing half of the premalignant lesional tissue pretreatment or in patients with normal epithelium, normal tissue was removed from the ventral lateral tongue. Patients were re-biopsied following the 6 week treatment, including the residual treated site and the initial biopsy area [17]. Endpoint measurements included histologic diagnoses and loss of heterozygosity at tumor suppressor gene loci (*INK4a/ARF, p53* and *FHIT*), which are altered in oral premalignancy [30] and associated with progression to squamous cell carcinoma [31, 32]. BRB administered as a berry gel for 6 weeks was well tolerated, as none of the patients reported adverse events and normal tissue epithelium from control patients remained normal [17]. Importantly, BRB gel treatment resulted in histologic regression in a subset of patients and resulted in a statistically significant reduction of loss of heterozygosity (LOH) prevalence [17]. Specifically, following BRB treatment 41% of subjects showed a decrease in lesional grade, 23% an increase in histologic grade and the remaining 35% no change in histologic grade [17].

Next further research was conducted in the same cohort, with 3 additional cases and expanded outcomes to include growth factor, proinflammatory and angiogenesis inducing enzymes (VEGF, COX-2, iNOS), gene expression profiles and microvessel density (MVD). Results showed that topical BRB gel suppresses genes associated with RNA processing, growth factor recycling, and inhibition of apoptosis [18]. In addition, COX-2 levels were significantly reduced post-BRB treatment [18]. BRB treatment reduced MVD, as measured by CD34, in 63.6% of patients; whereas, MVD levels increased in the remaining patients [18]. These results support that BRB gel has positive effects on histological regression, reduces LOH, inflammatory markers and MVD; however, there seems to be a subpopulation of patients that are more responsive compared to other patients [18].

3.2. Placebo controlled study of BRB gel in patients with oral premalignancy

Based on the promising results utilizing a BRB gel over a 6 week time period in patients with oral premalignancy, an expanded multi-centered placebo controlled trial of 3 months in duration was conducted to further assess efficacy [19]. Forty patients with microscopically confirmed oral premalignancy were enrolled, 22 patients were randomized to the 10% BRB gel treatment arm and 18 patients to the placebo gel arm. Topical application of BRB gel (0.5 g 4× daily) on oral premalignant lesions resulted in significant reductions in lesion size, histologic grade and LOH events. In contrast, lesions treated with placebo gel significantly increased in size without improvement in histologic grade or reductions in LOH levels [19]. Specifically, 70.6% of placebo treated lesions increased in size; whereas, 76.2% of BRB gel treated lesions reduced in size. Among BRB treated patients 41% experienced decreases in lesion grade following 3 months of treatment. The study further revealed higher pretreatment levels of BRB metabolic and keratinocyte differentiation enzymes in patient lesions that were BRB responsive, pointing to inherent differences in metabolic and differentiation capacity among patients [19]. As discussed by the author's interpatient variation in responsiveness pose a real challenge in the context of prevention trials [19].

Additional research is needed to improve our understanding of absorption, metabolism and bioactivity of BRB and their constituent polyphenols on a per patient basis. Kay and colleagues recently employed ^{13}C-labeling of cyanidin-3-glucoside (C-3-Gluc), a major BRB anthocyanin, as a novel approach to investigate pharmacokinetics and identify new metabolites following ingestion of C-3-Gluc [16]. Surprisingly, this targeted labeling approach revealed that anthocyanins have a minimum bioavailability of 12.4% on the basis of total elimination of absorbed ^{13}C dose [16]. This level is considerably higher than previous reports [33] and supports that anthocyanins are as bioavailable as other flavonoid subclasses. This study also reported high variation in the recovery of the ^{13}C tracer between subjects supporting large interindividual differences which in turn may impact compound fate and patient responsiveness to interventions, as noted in the oral cavity studies discussed [17–19]. New insight regarding absorption, metabolism and excretion can be derived from ^{13}C-labeling studies and should be considered for other polyphenols of interest.

Moreover, a highly accessible location like the oral cavity offers a ready model for investigating controlled direct delivery of agents and determination of interpatient response variability. It permits more precise delivery in terms of dose, ease of administration multiple times a day, likely requires less total product for efficacy and may benefit from local metabolism. Preclinical studies support that BRB have been most effective where there is some direct contact with the target area, with the only exception being a single *in vivo* study in a preclinical model for breast cancer [13]. Thus, novel labeling approaches may prove useful for improved targeting of inhibitory agents and increasing our understanding of differential patient responsiveness.

3.3. BRB in barrett's esophagus patients

Barrett's esophagus (BE) is the only known precursor lesion for esophageal adenocarcinoma (EAC), a rapidly rising cancer with poor survival rates [34]. Reflux of gastric and duodenal contents, known as gastroesophageal reflux disease (GERD), is the main risk factor for BE and EAC [35, 36]. GERD frequently manifests as heartburn

Table 1
Clinical trials of BRB targeting premalignancy or cancer

Population, no. patients	Route & mode of delivery	Dose	Duration of Study	Endpoints	References
Oral dysplasia ($n = 27$ and 30)	Direct local delivery in berry gel formulation ($n = 17$ and 20 w/ Premalignancy & $n = 10$ Controls)	0.5 g BRB or 10% w/w 4× daily	6 weeks	Lesion size Histopathology Loss of heterozygosity COX-2, iNOS, CD34 Gene expression	(17, 18)
Oral dysplasia ($n = 30$)	Direct local delivery in berry gel formulation ($n = 22$) or placebo ($n = 18$)	0.5 g BRB or 10% w/w 4× daily or placebo	3 months	Lesion size Histopathology Loss of heterozygosity COX-2, iNOS Gene expression	(19)
Barrett's esophagus ($n = 20$)	Oral consumption as Lyophilized black raspberries in water suspension	32 to 45 g BRB 1× daily	6 months	Cell proliferation Oxidative damage Lipid peroxidation Cholesterol GST-pi, CDX2, NF-κB Ellagitannin metabolites	(20, 21)
Colon Cancer ($n = 20$)	Oral consumption as Lyophilized black raspberries in water suspension	20 g BRB 3× daily (oral)	1–9 weeks	DNA methylation Cell proliferation Apoptosis Angiogenesis β-Catenin,E-Cadherin, c-Myc Cyclin D1 Metabolites	(22, 23, 24)
Familial adenomatous polyposis ($n = 14$)	Local delivery as rectal suppository alone ($n = 7$) or in combination w/ oral consumption of Lyophilized black raspberries in water suspension ($n = 7$)	1.4 g/d BRB suppository alone or in combination w/ 20 g BRB 3× daily (oral)	9 months	Polyp number, burden & size DNA methylation APC SNPs Cell proliferation	(25)

BRB, abbreviation for black raspberry.

and is estimated to impact over 60 million Americans. In addition, obesity imparts a 1.5 to 2.0-fold increase risk for BE and a 2 to 2.5-fold increase risk for EAC [36]. Thus, there is a large population at risk for BE and potential progression to EAC. Plant based diets rich in fruits, vegetables, and fiber are associated with reduced risk for EAC [1]. A 6 month pilot study was conducted to assess the long-term tolerability of a food based chemopreventive approach and to investigate whether BRB modulate oxidative damage and other aberrant

signaling cascades associated with GERD and progression of Barrett's esophagus. Details of the study approach and eligibility have been previously reported [20, 21]. In brief, all subjects were adults, 18 years of age or older with a diagnosis of Barrett's esophagus ($\geq$1 cm) on the current and two previous endoscopies. Twenty patients were enrolled with each essentially serving as their own control. Lyophilized or freeze dried BRB powder was administered at 32 and 45 g 1$\times$ daily to women and men, respectively. This gram quantity is approximately equivalent to 1.5 and 2 cups of whole fruit and was based on early preclinical research in animal models showing that 5 and 10% BRB in the diet inhibited esophageal cancer [5–9]. Patients mixed the BRB powder with about 6 ounces of water and consumed the mixture orally each morning. Urinary markers were assessed at baseline or pre-treatment and at 12 and 26 weeks post-BRB administration. Specific measurements included urinary excretion of 8-epi-prostaglandin F2α (8-PGF2α) and 8-hydroxy-2'-deoxyguanosine (8-OHdG), markers of lipid peroxidation and oxidative DNA damage. Urinary levels of the ellagitannin metabolites, Urolithin A-glucuronide, Urolithin A-sulfate and dimethylellagic acid glucuronide (DMEAG) were also investigated for the first time, as potential markers of compliance. Esophageal and gastric tissues were assessed for changes in markers linked to proliferation, differentiation, detoxification and inflammation. Specific immunohistochemical markers assessed in esophageal tissues included Ki-67, CDX2, GST-pi, and NF-κB. In addition, secondary outcomes included changes in histopathology, Barrett's esophageal length, blood pressure, total cholesterol levels and body mass index [20, 21].

Findings from this study showed that BRB were well tolerated at 32 and 45 g/daily for 6 months and that compliance was high with 96% consuming BRB daily, based on intake records and counts from returned packages. Levels of the ellagitannin metabolite, Urolithin A-glucuronide, significantly increased following BRB treatment for 12 and 26 weeks compared to baseline [20, 21]. At baseline 15% of patients expressed detectable levels of Urolithin A-glucuronide [70 ng/mL]; whereas, 85% of patients expressed elevated levels at week 12 [1287 ng/mL] and week 26 [1723 ng/mL] of study supporting that urolithin A-glucuronide may serve as an additional indicator of compliance. BRB consumption resulted in elevated levels of urinary urolithin A-sulfate and DMEAG; however, these metabolites were not impacted to the same degree or magnitude [21]. Differences in metabolizing enzymes may be responsible for variations in response among patients, but additional research is warranted to better understand the role of individual differences in metabolizing enzymes and potential interaction with the microbiome profile of individual patients.

BRB consumption reduced urinary excretion of 8-PGF2α following 12 weeks, reaching statistical significance by 26 weeks of treatment. This marker is considered a reliable and relatively stable marker of overall oxidative stress levels and more specifically an indicator of lipid peroxidation. Reductions in 8-PGF2α are especially promising, considering that average BMIs increased significantly over the 6 month trial. BRB consumption did not significantly reduce levels of 8-OHdG, a marker of DNA damage and there was greater variability noted for this marker with increases noted among 10 patients and decreases among 6 patients over the study duration [20, 21]. Interestingly, total cholesterol levels non-significantly declined in BRB treated patients, particularly those who presented with baseline cholesterol levels > 200 mg/dL ($p = 0.165$). Cholesterol levels reduced from 234 mg/dL at baseline to 226 mg/dL at week 26, following BRB treatment [21]. The latter finding is in alignment with other recent studies pointing to the positive effects of berry consumption on indicators of cardiovascular health, even in patients with metabolic syndrome [37]. Jeong and colleagues recently reported that a BRB extract administered to patients with metabolic syndrome significantly reduced total cholesterol and cytokines including IL-6 and TNF-α levels following 12 weeks of consumption [37]. Obesity is a strong risk factor for both BE and EAC [35, 36]; thus, agents which positively impact risk factors, as well as defined cancer processes hold particular promise. In terms of tissue specific changes, the main positive effect was increased expression of the detoxification marker GST-pi in BE epithelium of 55.6% of patients. Overall proliferation rates as measured by Ki-67 were unchanged with decreased levels noted in two patients and increased levels in 2 other patients [21]. There were no significant changes in BE histopathology grade or length of the BE tongue following treatment. However, it is important to note that detection of a 30% or 1 cm change in the length of the BE segment would require a patient sample size of 43; thus, this pilot study was under powered for detecting changes in BE length.

Taken together, these results support that BRB consumed at 32 to 45 g/day positively impact plasma metabolites including total cholesterol and increased urinary ellagitannin metabolites, reduced a urinary marker of lipid peroxidation, and importantly at the tissue level significantly increased GST-pi levels, a marker of detoxification [21]. Potentially higher doses, more frequent administration or an alternative delivery matrix is required for modulation of additional tissue specific markers.

3.4. BRB in colorectal cancer patients

Colorectal cancer (CRC) is the second leading cause of cancer related deaths with over 49,000 cases expected in the US this year [34]. Over the last 20 years improvements in screening and treatment modalities have led to decreases in the colorectal cancer death rates. Despite these advances approximately half of patients diagnosed with CRC will eventually die of the disease [1, 2]. Thus, there remains a critical need for more effective preventive and treatment options targeting colorectal cancer. It is estimated that 50% of colorectal cancers could be prevented through exercising and maintaining a healthy weight, limiting consumption of red and processed meat and eating a plant based diet rich in vegetables, whole grains and fruit [1, 4]. Positive preclinical results support that dietary BRB significantly inhibited the formation of chemically induced colon tumors [10, 11]. A clinical trial was initiated enrolling 20 colorectal cancer patients. Exclusion and inclusion criteria were previously published [22–24]. All patients had tissues taken before and after 1 to 9 weeks of oral BRB (20 g 3× daily) consumption. Changes in markers associated with cell proliferation, apoptosis, angiogenesis and Wnt signaling were measured. Positive findings were noted in patients who consumed BRB for 4 or more weeks. Specific findings included BRB altered methylation of DNMT1, SFRP2, and PAX6a in normal adjacent tissues and DNMT1, SFRP2, PAX6a and WIF1 in colorectal tissues [22–24]. BRB did not induce changes in global methylation. Immunohistochemical staining results showed that BRB modulated β-catenin, Ki-67, TUNEL, CD105, and DNMT1 in colorectal tissue and CD105 and DNMT1 in normal tissues [22–24]. The fact that BRB significantly altered a number of markers in key molecular pathways known to be altered in colon cancer is encouraging and it is notable that the effect occurred in a relatively short period of time. Positive effects were found for many of the outcomes in patients who had been consuming BRB for 4 or more weeks supporting that even short-term or potentially intermittent use of agents with cancer inhibitory potential may offer benefits.

Research findings from the above colorectal trial were extended to include BRB induced modulation of select plasma cytokines [23] as well as metabolic profiles in the plasma and urine following treatment [24]. Significant changes in plasma levels of GM-CSF and IL-8 were reported in patients consuming berries for more than 10 days and changes correlated with apoptosis induction and reduced proliferation in colorectal tissues [23]. These results support that BRB induce changes in circulatory markers rather rapidly (10 days), but that tissue specific alterations require a longer duration of BRB administration (4 or more weeks) or higher cumulative dose. These results are consistent with those from the BE study [21]. A non-targeted metabolic analysis uncovered over 400 annotated metabolites, with 34 and 16 metabolites significantly changed by BRB in the urine and plasma, respectively [24]. Specific metabolic pathways altered with BRB treatment include amino acid metabolism, lipid metabolism and xenobiotics. BRB polyphenols were metabolized to multiple benzoate species which was associated with an enhanced amino acid metabolite. Further, increased levels of 4-methycatechol sulfate in both urine and plasma correlated to increased apoptosis at the tissue level [24].

3.5. BRB in patients with familial adenomatous polyposis

Familial adenomatous polyposis (FAP) is an inherited disorder characterized by early onset of colonic polyposis and progression to cancer of the colon and rectum by age 40 [39, 40]. FAP is due to germline mutations in the *APC* gene and requires patients to be closely monitored via endoscopic surveillance for early detection of polyps. Once polyps manifest the only effective management to prevent cancer is eventual colectomy; thus,

agents that delay polyp progression may offer alternative or complementary preventive options. Non-steroidal anti-inflammatory drugs (NSAIDS) regress FAP associated polyps, but due to associated gastrointestinal, cardiovascular and cerebrovascular events non-toxic alternative strategies are being investigated. Thus, a small pilot study enrolled 14 patients with FAP to assess the potential beneficial effects of BRB on measurements of polyp number, size, and burden. BRB were administered daily for a period of 9 months. Arm 1 consisted of 7 patients consuming a maltodextrin placebo powder three times a day combined with two BRB rectal suppositories (0.7 g each administered at bedtime). Arm 2 included the remaining 7 patients who consumed freeze dried BRB powder (20 g 3 $\times$ daily) mixed with water, plus two BRB rectal suppositories (0.7 g each administered at bedtime). Study specifics have been previously reported [25]. In brief, inclusion criteria included: $\geq$18 years of age, a diagnosis of FAP with at least 5 rectal polyps $>$ 2 mm at baseline endoscopy and no NSAID use currently or in the last 2 months. Prior to treatment initiation and at 36 weeks of study each patient underwent flexible sigmoidoscopy to record polyp number, size, and burden defined as the sum of diameters of all adenomas $>$ 2 mm. Up to two polyps were removed at baseline for biomarker analysis and all polyps were harvested at 36 weeks of study. Additional outcomes includedimmunohistochemical analysis of Ki-67 as a marker of cellular proliferation, TUNEL staining for apoptosis, cMyc, DNMT1, p16 and analysis of DNA methylation [25].

Combining the two treatment arms, study results showed a significant reduction in tumor burden post-treatment with BRB suppositories [25]; however, the authors concluded that there was no additional benefit for patients consuming BRB orally. Interestingly, the magnitude of change from baseline polyp burden does appear greater given the combined treatment (about 75%) compared to suppositories alone treatment (about 40%), raising the question of whether there is a benefit in terms of magnitude of effect. It is likely that a sample size greater than 7 is needed to assess whether combined oral consumption of BRB with suppository treatment offers any increased efficacy compared to suppositories alone. Certainly, as discussed in the context of trials targeting oral premalignancy, the use of suppositories may offer the advantage of local delivery depending on polyp location; however, expanded studies are necessary to determine whether combined oral and direct delivery approaches offer any advantages for FAP patients. Three patients presented with increases in polyp number and burden following treatment completion; one patient in the suppository alone arm and two patients on the combined treatment arm [25]. Patients that responded to BRB treatment as evidenced by decreased tumor burden also showed reductions in cell proliferation rates as measured by Ki-67, decreased DNMT1, increased P16 levels, yet, cMYC and TUNEL expression were reportedly unchanged by BRB treatment [25]. BRB treatment resulted in decreased *p16* promoter methylation, but did not differentially impact Wnt pathway antagonists, *SFRP2* and *WIF1* in polyps from responders versus non-responders. Responders were also investigated for differences in somatic SNPs in the APC gene, but no differences were detected [25].

These results are encouraging and in alignment with earlier research conducted in colon cancer patients in terms of p16 findings, but unique in that Wnt regulators were not differentially altered between responders and non-responders [25]. Still, this study did detect that miRNAs regulating Wnt pathways were demethylated by BRB in adenomas from responders. Collectively, the data utilizing BRB to target colon cancers is highly positive showing inhibitory effects in both inherited FAP associated and non-FAP associated colon cancers. Once again, the results support that the cohort of FAP patients appeared to have patients that responded to BRB exposure and a subpopulation of non-responders.

4. Discussion/Conclusion

Evidence between single food sources and cancer protection is limited and challenging to study. To date, the strongest cancer inhibitory evidence exists for food groups and especially plant based diets rich in fruits, vegetables and fiber [1]. In terms of extracts, much of the evidence for cancer protection is strongest the closer the extract mimics the whole plant components [42–44]. To determine the esophageal cancer inhibitory contribution made by BRB versus BRB derived fractions, a preclinical *in vivo* study was conducted in F344 rats comparing a 5%

lyophilized black raspberry powder to a BRB derived anthocyanin rich fraction, a BRB derived ethanol/water soluble fraction, a BRB derived insoluble fraction, a BRB derived hexane extract and a BRB derived sugar fraction [42]. The results supported that the 5% BRB, the anthocyanin fraction and ethanol extract which all contained similar levels of anthocyanins were equally effective in inhibiting NMBA-induced esophageal cancer [42] supporting the historical rationale that anthocyanins are the cancer inhibitory component of BRB. However, the BRB derived insoluble or residue fraction was nearly as inhibitor and this fraction contains very low levels of anthocyanins [42] illustrating the complex nature of individual food products and the fact that many foods of interest are not fully characterized from a cancer inhibitory standpoint.

A clinical investigation evaluating red and yellow tomatoes versus similar levels of lycopene in the form of a purified extract reported short term tomato intake induces serum changes that favorably modulate cancer-related gene expression profiles in treated cell lines, including the yellow or lycopene-free tomato; whereas, purified lycopene had both positive and potentially deleterious effects as evidenced by up-regulation of pro-cancer genes [43]. Thus, identification of the main or sole component from a fruit or food matrix responsible for cancer inhibition remains challenging despite technological advancements.

Still, the combination of preclinical and early clinical studies utilizing various black raspberry products have revealed beneficial effects linked to cancer inhibition in select targets as summarized herein [17–25]. Completed published studies evaluating BRB in the clinical setting report positive effects on preneoplastic lesions or cancers of the head and neck, esophagus and colon. The positive effects of BRB in clinical evaluations include: anti-proliferative effects; activation of pro-cell death pathways; histologic regression of oral intraepithelial neoplasia associated with improved histologic grade and significantly reduced LOH at tumor suppressor gene associated loci, reduced suppression of genes linked to RNA processing, growth factor recycling, and reduced COX-2 levels in the oral cavity; in the colon, inhibition of FAP-associated polyp progression, demethylation of known tumor suppressor genes (Wnt pathway antagonists and p16) and improved plasma cytokine profiles (GM-CSF, IL-8) were reported; in Barrett's patients, increased tissue levels of GST-pi, decreased lipid peroxidation/oxidative stress (urinary 8-isoprostane levels) and reduced cholesterol levels followed BRB treatment. In addition, other studies of obese patients have reported improved serum cytokine levels and lipid profiles including IL-6, TNF-α, and cholesterol [34]. Results have not all been uniform across studies or even within, most studies report a percentage of responders and non-responders within the cohort evaluated, likely reflecting the heterogeneity between subjects and the complexity of each cancer or precursor lesion. In addition, the mode, delivery matrix, concentration, frequency and duration of black raspberry delivery has varied across studies, potentially contributing to divergent outcomes. BRB have been administered as a freeze dried powder suspended in water prior to consumption (37 to 60 g/day), as a rectal suppository alone (1.4 g/day) or in combination with oral delivery (60 g/day) and as a topical agent (10% w/w) delivered in a bioadhesive gel in the oral cavity. Studies conducted in patients with FAP or oral premalignancy permit relative direct delivery of BRB bioactive constituents to the target area. This has the advantage of requiring less product, increasing direct contact time and takes advantage of local metabolism compared with oral delivery of the suspended freeze-dried powder which allows less direct contact and relatively more systemic benefits. Still, common themes across studies have emerged including that BRB have anti-inflammatory effects, reduce oxidative stress, impact metabolism and restore tumor suppressive activity in a target specific manner resulting in regression of oral cavity and colon lesions.

The precise dose and duration of BRB required for optimum cancer inhibitory effects remains to be elucidated, but results to date suggest lower concentrations may favorably impact lipid profiles, while potentially higher concentrations are required for tissue specific effects. In addition, the lengths of studies have ranged from 1 week to 9 months with data supporting that longer duration studies of at least 4 weeks to 3 months or beyond have greater impact. Further research is warranted to increase our knowledge of BRB cancer inhibitory mechanisms and to improve our ability to discern which patient populations are likely to benefit from BRB administration. A marker of compliance to reliably link intake to effects would be valuable in future clinical trials as well. Ellagitannin metabolites were included in the BE trial, but were expressed in only 85% of the BE patients illustrating the complexity and limitations of the approach of utilizing metabolites which may vary among

patients due to inherent genetic or metabolic variation. An alternative approach that should be considered in future prospective trials is to add a food grade standard to BRB products permitting more accurate compliance measurements.

Progress has been made with BRB as well as other polyphenols in terms of understanding bioavailability and metabolism, but additional work is needed. Kay and colleagues utilized 13C- labeling approach to evaluate absorption, metabolism and excretion of cyanidin-3-glucoside in blood, urine and fecal samples following administration of a single 500 mg dose. This study uncovered a number of new metabolites offering improved understanding of compound fate. Additional studies incorporating these approaches with additional polyphenol metabolites, in varied matrices and following longer periods of administration would be highly beneficial. In addition, there is interest and active research focused on developing novel products for delivering food-based agents targeting cancer prevention which may offer alternative modes or matrices of delivery, especially to highly accessible targets. As an example, pectin and starch based black raspberry confections retain 93% of original anthocyanins compared to only 60% in a hard candy confection designed to delivery phytochemicals [45].

In conclusion, human clinical trials utilizing BRB have demonstrated chemopreventive effects in the oral cavity, esophagus and colon. However, additional research is warranted and should include 1) assessments of other more readily available berries that have demonstrated beneficial health effects, alone or in combination with BRB; 2) comparison of the whole BRB fruit versus dominant constituents and active metabolites; 3) optimizing BRB delivery mode and matrices; 4) factors impacting BRB metabolism and bioavailability [46, 47]; 5) effects BRB on cancer-initiating cells or cancer stem cells [48]; 6) BRB treatment as it relates to phase of carcinogenesis; and 7) whether BRB improve chemotherapeutic or radiation efficacy.

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