Title

Designing a broad-spectrum integrative approach for cancer prevention and treatment

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Abstract: Targeted therapies and the consequent adoption of "personalized" oncology have achieved notable successes in some cancers, however significant problems remain with this approach. Many targeted therapies are highly toxic, costs are extremely high, and most patients experience relapse after a few disease-free months. Relapses arise from genetic heterogeneity in tumors, which harbor therapy-resistant immortalized cells that have adopted alternate and compensatory pathways (i.e., pathways that are not reliant upon the same mechanisms as those which have been targeted). To address these limitations, an international task force of 177 scientists was assembled to explore the concept of a low-toxicity "broad-spectrum" therapeutic approach that could simultaneously target many key pathways and mechanisms. Using cancer hallmark phenotypes and the tumor microenvironment to account for the various aspects of relevant cancer biology, interdisciplinary teams reviewed each hallmark area and nominated a wide range of high-priority targets (83 in total) that could be modified to improve patient outcomes. For each target, a corresponding low-toxicity therapeutic approach was then suggested; many were phytochemicals. Proposed actions on each target and all of the approaches were further reviewed for known effects on other hallmark areas and the tumor microenvironment. Potential contrary or pro-carcinogenic effects were found for 3.5% of the relationships between targets and other hallmarks, and mixed evidence of complementary and contrary relationships was found for 7.8%. Approximately 67% of the relationships revealed potentially complementary effects, and the remainder had no known relationship. These results suggests that a broad-spectrum approach should be feasible from a safety standpoint. This novel approach has potential to help us address disease relapse, which is a substantial and longstanding problem, so a proposed agenda for future research is offered.

February 21, 2015

Dr Anupam Bishayee
Guest Editor
Seminars in Cancer Biology

Dear Dr Bishayee,

We are pleased to submit a revised version of the manuscript, 'A Broad-Spectrum Integrative Design for Cancer Prevention and Therapy."

A substantial number of changes have been made in the manuscript and changes in the authorship have also been made. Notably, Mr Leroy Lowe is now designated as a co-corresponding author. Mr Lowe is the architect of the Halifax Project. He was a major force in the conceptualization of the project's scientific basis, and was responsible for the organization of the project and coordination of the entire review process as well as organization of the project meeting in Halifax in 2013. He has made numerous organizational and technical contributions to the capstone manuscript itself. Corresponding authors are frequently asked to discuss publications with media representatives. Mr Lowe will be in an excellent position to do this, because of his deep involvement with the project and his thorough knowledge of the activities of all those on the hallmark review teams. In addition, Dr Brooks Robey has been added as a coauthor. He is a member of the carcinogenesis project, and wrote a paragraph on the limitations of the cross-validation project for the carcinogenesis group. At the request of a reviewer interested in this topic, the paragraph written by Dr Robey was added, nearly verbatim, to the Methods section (please see Response to Reviewers, Reviewer 3, Comment 3 for the paragraph in question).

Two versions of the manuscript have been submitted. The first version, Capstone revision 2 20 2015 showing changes.doc, shows changes made to the manuscript. Added sections are highlighted and deleted sections are crossed out; per the editor's instructions, Word Track Changes was not used. The second version, Capstone revision 2 20 2015 clean copy.doc, shows all the needed changes implemented in the manuscript. The instructions on manuscript quality control have been followed, with one exception. Since there are so many coauthors, we contacted the journal and received permission to use superscripted numbers rather than letters to link the coauthor names to their affiliations.

The manuscript has been shortened. Rather than ending on page 47, as in the original version, the text of the manuscript now ends on page 44.

Responses to Editorial Comments:

Comment 1. *The title of your manuscript may be appropriate for a short editorial (one to two-page) for this theme issue. Hence, it is requested that you may consider a slightly different title in line with the present one.*

Response 1. We have discussed this suggestion at length and believe that we should create a title for the introductory editorial that speaks more directly to the problem and the way it can be approached – as follows:

"Extending the principles of targeted therapy to tackle heterogeneity in refractory cancers"

We think that this title will resonate with a very broad audience because refractory cancers are the Achilles heel of the field and targeted therapy is the standing paradigm. If this isn't perfectly suitable, we can discuss variations on this theme but we do want to focus on the problem in the title of the introductory editorial.

If we take that approach, then we can use the existing title for the capstone paper which more appropriately speaks to the fully conceived solution.

We hope you agree with this approach.

Comment 2. *Pages 20-32 (various hallmarks of cancer): It is recommended that this section should be either completely deleted or greatly condensed avoiding text which may be used in identical form in other review manuscripts on these hallmark areas. There are instances where the text may not be appropriate in the way it is presented, e.g., (page 30, last paragraph) "In our review…….cancer". Obviously, the authors are referring to other resources without any citation.*

Response 2. The hallmarks review section has been completely rewritten. Each hallmark has been shortened, and in addition, at the request of a reviewer, the targets and approaches selected for each hallmark have been listed in the abstracts. We examined the hallmark sections for inappropriate text and did not find any: the words that you mention on page 30 were removed. Rather than showing the original material crossed out, we simply deleted the entire hallmarks section and replaced it with the new section, which is highlighted in yellow.

Comment 3. *There have been numerous inaccuracies with reference formatting, e.g., page 22, last paragraph: it should be [105-107]. Please check the entire manuscript and correct similar bibliographic errors.*

Response 3. We have gone through the manuscript and removed formatting errors.

Comment 4. *Abstract:*

WE EMPOWER PATIENTS TO BECOME NUTRITIONALLY, PHYSICALLY AND PSYCHOLOGICALLY FIT TO BETTER FIGHT CANCER

a) Line 9: Please consider rephrasing: "….many cell growth and death pathways"

b) Line 12: Please consider "combining" in place of "grouping".

c) Line 14: Please use "..abnormal signaling pathways".

d) Line 16: Please consider using a substitute for "collaborative" e.g., cadre.

Response 4. The abstract has been completely rewritten to make it shorter. Most of these comments are no longer relevant. We substituted "task force" for "collaborative."

Comment 5. *Various sections and subsections of the manuscript should be numbered as per the style of the journal.*

Response 5. The section and subsection numbering now matches the journal style.

Comment 6. *Please remove figure 1 from the manuscript and submit it separately.*

Response 6. Figure 1 is submitted separately. We have added a second figure at the suggestion of one reviewer, which is also submitted separately.

Comment 7. *Please move the legend for Figure 1 at the end of the manuscript (before references).*

Response 7. Legends for both figures are at the end of the manuscript, before the references.

Comment 8*. Page 13, line 4: Use "nuclear factor-kB (NF-kB). For all subsequent use, just use the acronym.*

Response 8. The abbreviation rule has been followed throughout the manuscript.

Comment 9. *Page 14, line 5: Insert a reference number after "Vogelstein et al."*

Response 9. The reference was inserted.

Comment 10. *Page 18, third paragraph: Ref. 55 should not be in superscript.*

Response 10. The reference format was corrected.

Comment 11. *Please place tables at the end of the manuscript (each starting on a new page), not embedded in the text.*

Response 11. Tables are placed at the end of the manuscript.

Comment 12. *Page 45, line 17: insert "and" between last two plants.*

Response 12. This correction was made.

Comment 13. *The bibliography section should adhere to the style of the journal. The reference number should not be in superscript and doi numbers may be deleted.*

Response 13. The reference format was corrected and doi numbers were deleted.

Please notify me if you have any further questions about the manuscript. We look forward to the completion of the review process.

Sincerely,

Keith

Keith I. Block, MD

Medical Director, Block Center for Integrative Cancer Treatment

***Revision Notes**

Reviewer 1

Comment 1. *This article provides abundant support for a broad spectrum approach to cancer therapy that is based on a clearly delineated framework. The strength of the article is in its articulation of a novel paradigm for an approach to cancer therapy. However, it does not provide clear guidance for the practical application of this paradigm. Perhaps this is not the intent for as has been clearly noted, this effort is at an early stage. In that case what follows is a justification for the broad spectrum approach to cancer prevention and therapy. In this regard, it succeeds very well with clear reference to elements of the framework, in particular the Hallmarks of cancer, pathways of progression and therapeutic approaches and rigorous justification for each in the context of the overall model.*

Response 1. The authors thank the reviewer for the supportive comments regarding the overall model. The paper is not focused on practical applications but on establishing the framework of the model, with some general comments on potential research and development. Because of the length and detail involved in presenting the hallmark summaries and the cumulative results of the cross-validation process, it is simply not possible to go into great detail on the practical application of the broad-spectrum approach. The validation of a rather minimal number of "contrary" interactions of the targets and approaches with hallmarks, and the affirmation of a large amount of unknown relationships, establishes a theoretical basis for pursuing further research. The Proposed Research Model section does lay out some guidelines for research. However, a detailed guide to practical implementation of this research is beyond the scope of the paper. See also our response to question 3 of Reviewer 1 below.

Comment 2. *The article appears to be a consensus document that describes the contributions of multiple groups using agreed upon methods that in places appear to be more of a mosaic of concepts rather than a seamlessly integrated discourse. Again, this is a very daunting task given the number of groups and authors contributing to this important effort. Given the challenges, I would like to suggest some recommendations (not requirements) that may help the presentation of these concepts. One suggestion is to frame the article as a consensus document for a broad spectrum integrative design for cancer prevention and therapy. Following the introduction which summarizes the framework of the model, the following sections should be viewed as justification for each element of the model as determined by each group (much of which is a literature review). More liberal use of diagrams to visually convey important principles of the model would be very helpful and welcome.*

Response 2. We agree that the nature and structure of the article as originally formulated was difficult to frame for the readers. While this is not a formal consensus document, it is a capstone for the work of the Halifax project, and was iteratively circulated to all coauthors for comments, inputs and feedback. It is also the final article in a special issue composed of a series of reviews of eleven cancer hallmarks with a view towards developing the broad-spectrum approach. So it does represent the intent of all of the authors and it provides a scientific framework and research model for the broad-spectrum approach that is based on these reviews and the discussions that took place amongst the project participants. To better convey this shared perspective, we have revised a paragraph in the introduction, which we intend to give a more understandable explanation of the nature of the project and this capstone paper.

Furthermore, to address the suggestion of additional diagrams, we decided that the figure that was most needed was in the section in which the hallmark summaries are provided. We therefore provide Figure 2, shown below the introductory paragraph, to show and explain the hallmarks of cancer model for those unfamiliar with it. Although we considered additional depictions for each of the hallmark areas, we believe that this single diagram will serve as an overview for the reader and help to visually convey the basic framework. It was our concern that additional depictions of indepth biology would be a distraction from the strategic and holistic perspective that we are trying to convey.

Paragraph in introduction (page 9 in clean copy):

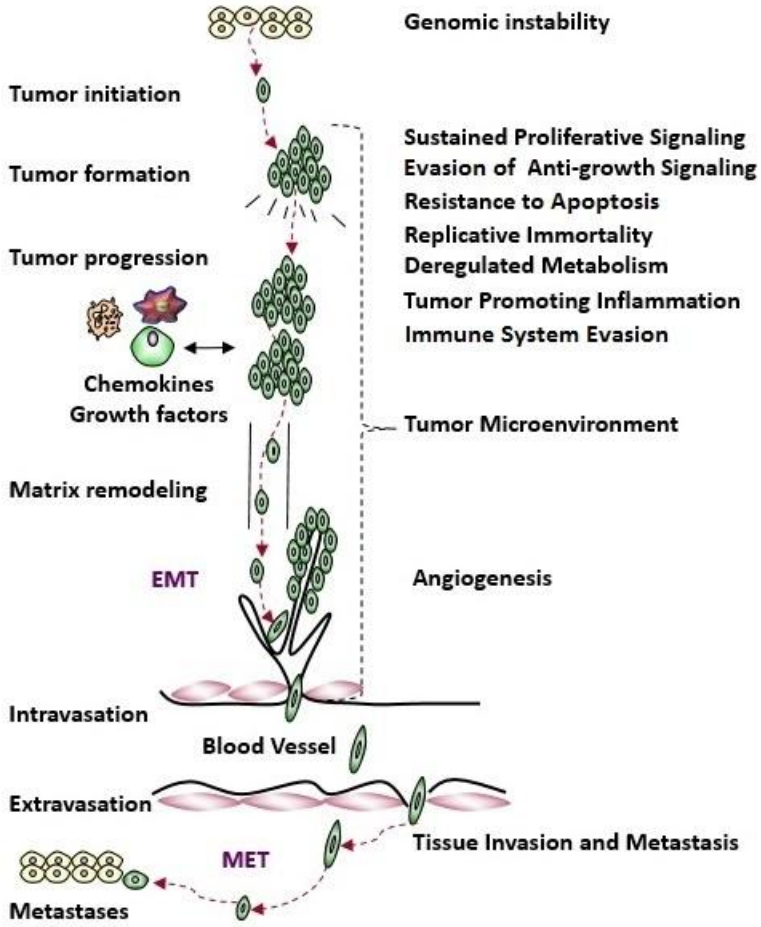
> This capstone paper describes the methods and results of a substantial effort by a large international group of biochemical and medical researchers, operating under the name of "The Halifax Project," sponsored by a non-profit organization, Getting To Know Cancer It summarizes and draws together material from a series of reviews on the hallmarks of cancer, presented in this special issue of Seminars in Cancer Biology, to present a conceptual framework for a new approach to cancer prevention and therapeutics. This approach involves the targeting of many high-priority anti-cancer mechanisms and pathways within a more comprehensive model of treatment and care. We refer to this as a "broad-spectrum" approach (i.e., an approach aimed at a broad spectrum of important mechanisms and pathways). The approach involves combinations of multiple low-toxicity agents that can collectively impact many pathways that are known to be important for the genesis and spread of cancer. By making extensive use of chemicals from plants and foods that have already been studied or utilized for cancer prevention and treatment, this approach offers a compelling rationale for addressing the underlying biology of cancer while being efficacious, non-toxic and cost-effective. We come together in the belief that a broad-spectrum approach of this type, in the context of a clinical environment including conventional treatment and attentive to optimal health, would provide real benefit for cancer patients. In this paper we describe the rationale for broad-spectrum therapeutics, detail the methods of the Halifax Project, summarize potential targets and agents related to eleven hallmark features of cancer, propose a research model for the development of broad-spectrum therapies, and call for action to advance this research model.

Text describing Figure 2 (beginning of hallmark summaries section, page 22 of clean copy)

> The hallmark summaries are roughly sequenced to capture the acquired capabilities of most cancers (see Figure 2). The section begins with *genomic instability*, an enabling characteristic, followed by *sustained proliferative signaling* and *evasion of anti-growth signaling*, two hallmarks that ensure that proliferation is unabated in cancer cells. These are followed by *resistance to apoptosis* and *replicative immortality*, two layers of defense that are believed to be bypassed in all cancers. Then we discuss *deregulated metabolism and tumor-promoting inflammation*, which signal an important self-reinforcing evolution in the tumor microenvironment. Sections on *angiogenesis* and *tissue invasion and metastasis* speak to disease progression.

Finally the *tumor microenvironment* and *immune system evasion* summaries relate to the last lines of defense to be defeated in most cancers.

Figure 2. Hallmarks of cancer, sequenced roughly in the order in which these capabilities are acquired by most cancers, as portrayed in the graphical representation of tumor evolution.



Comment 3. *While the efforts of the contributors clearly supports the credibility of the approach and again acknowledging seeks to provide a basis for future research and clinical interventions , a concluding section that at least begins to integrate attributes of the model into a practical set of consensus guidelines would be invaluable. There are likely many practitioners that may have difficulty getting beyond the necessary and important details who could benefit from a practical illustration of the principles in the form of guidelines. In this way the document could be viewed as a consensus approach and guidelines for a broad spectrum integrative design for cancer prevention and therapy.*

Response 3. Thank you for raising the issue of relevance to clinicians. This paper is not truly intended for clinicians, but there is little doubt that it will be of interest to them. We have therefore inserted a new paragraph in the section Summary and Conclusions, as shown immediately below. It is the feeling

of the group that an evidence-based clinical application of the broad-spectrum approach is premature – especially since the basic research that we describe in the Proposed Research Model section truly is necessary before we can claim to be supplying a useful broad-spectrum therapeutic. However, we can point out that integrative lifestyle therapies are closest to being appropriate for clinical use at this point, and that they do address a large number of relevant topics, but also that uninformed use of unresearched phytochemical combinations in treatment is premature.

Statement in Summary and Conclusions (page 42 of clean copy)

> What are the implications of this broad-spectrum strategy for current clinical practice? First, clinicians should realize that this paper presents a developmental research program, not clinical guidelines. Use of uninformed selections of phytochemical or botanical extracts in poorly-defined clinical situations is unlikely to deliver positive results. Further, as noted above, concerns with interactions of natural products with conventional treatments should be kept in mind. That said, lifestyle therapies appear to affect multiple molecular targets and to improve the health of cancer patients in a variety of ways [34,148]. Clinical trials are defining beneficial impacts of natural products [247]. The positive implications of dietary therapies for improvement of the metabolic hallmarks of inflammation, deregulated metabolism, genomic instability and immune system evasion should be kept in mind [248,249]. Clinicians choosing to use natural product supplements should attend to product quality and be familiar with advances in the formulation of poorly absorbed polyphenols and other phytochemicals [199-201].

Comment 4. *One specific area that deserves more attention is the role of clearly defined botanical extracts and their effects of hallmarks and not only single molecules derived from various botanicals such as curcumin and EGCG. There is ample evidence in the literature to suggest a role for rigorously defined botanical extracts in the context of this model. Furthermore, potential mechanisms for these extracts which may involve inherent synergies has already been alluded to in the article by the acknowledgement that many natural products are efficacious, while lacking sufficient blood or tissue concentrations (based on in vitro research) to explain the efficacy. This may help to make an important connection to use of botanicals in traditional medicine systems such as Ayurveda and traditional Chinese medicine. Of course the broad spectrum approach is not limited to botanicals since other practices have been shown to modulate important targets.*

Response 4. Thank you for pointing out the role of botanical extracts. The emphasis on phytochemicals for the review teams did result in too little attention paid to defined botanical extracts (in addition to defined food extracts, as pointed out by another reviewer). While some discussion of these exists in the manuscript already, we added a sentence to the Summary and Conclusions section under the discussion of the pharmacology of mixtures (page 43 of clean copy):

> And although this effort emphasized phytochemicals, it is also important and relevant to study defined botanical and food extracts. Standardized black raspberry extract, for instance, has produced positive results in human trials on apoptosis, angiogenesis and several specific targets selected in the project. [253]. Aged garlic extract [254] improved immunity in advanced cancer patients, and lyophilized strawberries [255] improved

premalignant esophageal lesions. Defined herbal extracts such as PHY 906 and BZL101 mentioned above have demonstrated preliminary antitumor activity [218,219].

Comment 5. *In summary, this article embodies an excellent justification for a broad spectrum and integrative approach to cancer prevention and therapy and also research that may benefit from some of the hitherto mentioned suggestions.*

Response 5. Thank you for the supportive comment, and we hope that our responses above are helpful.

Reviewer 2

Comment 1. *Recommend shortening the manuscript. I think, part of the manuscript will be repeated in the accompanied manuscripts.*

Response 1. We agree that this manuscript was too long so we have shortened it in the following ways: (1) each of the summaries of the hallmarks was shortened and made more uniform in length, content and number of references, and (2) the section on regulation in which regulations of several different countries were discussed in some detail was collapsed into a single paragraph. In the copy of the paper showing changes, rather than showing the deleted material in cross-outs in these sections, we have simply replaced it in the text. In addition, smaller edits were made throughout the manuscript to eliminate unnecessary paragraphs or words and redundant content. Other reviewers requested the addition of new material, but we believe that overall this streamlined version of the manuscript is now a much more suitable length.

Comment 2. *Additional activity of phytochemicals is in reducing side effect of many chemotherapeutics. Furthermore, natural product may sensitize activity of chemotherapeutic agents. Such activities needed to be included.*

Response 2. These are important features of natural products. We have added two sentences on this topic to the section on Clinical Considerations (page 40):

> Curcumin is one of several natural products that act as chemosensitizers and radiosensitizers for several tumors, while protecting normal tissues [230]. The ability of herbs and other natural products to relieve treatment-related side effects should not be overlooked [231,232].

Comment 2. *Imatinib is an inhibitor of Abl tyrosine kinase and not BCR (Page 7, lane 38).*

Response 2. Thank you for pointing out this error. It has been corrected.

Comment 3. *References should listed in one format (some contain DOI and other not). Follow instructions of the Journal.*

Response 3. Thank you; the necessary corrections have been made.

Comment 4. *Recommended to include clinical resistance in cancer such as resistance in APL to RA in the general topic of "Resistance to apoptosis" (Page 25, lane 20).*

Comment 5. *Resistance to apoptosis also related to CSC. Need to address this point (Page 25).*

Response to 4 and 5. As part of the overall shortening of the paper, the apoptosis section was shortened. The apoptosis team acknowledges the importance of these two topics but did not feel there was room for them in the shortened apoptosis summary section.

Comment 6. *Correct 5many (Page 43 , lane 20)*

Response 6. Thank you for noticing this; the correction has been made.

Comment 7. *Add space in "toolkit" (page 44, lane 17).*

Response 7. Thank you; the correction has been made.

Comment 8. *Table 2 contains list of natural products and targeted therapy agents. Need to separate them*

Response 8. This is an interesting suggestion that was discussed at length by the many scientists who attended the Halifax Project workshops. The directive to the hallmark teams was to find low-toxicity and relatively low-cost approaches, without specific direction as to whether they were natural, synthetic or targeted agents (emphasizing natural agents when feasible). Our team discussed this and we believe that the ability to easily look up potential agents, since they were in alphabetical order, was a practical consideration against the change. We also believe that making a separation between the natural and other agents introduces and implies a somewhat greater importance to the natural compounds than they actually had in the selection process. However, we are sensitive to the interest in the difference between natural and synthetic or targeted agents. We have thus indicated the synthetic and targeted agents in the table with double asterisks, which preserves the alphabetical order but designates the different types of compounds.

Reviewer 3

Comment 1. *This manuscript provides a framework for a special issue of Seminars in Cancer Biology that describes the outcomes of a large working group considering ways to optimize integrative oncology*

through the rational inclusion of nutritional, exercise habit and biobehavioral approaches in conjunction with current therapeutic approaches to precision oncology.

Overall, the article is very well written. The rational for convening a working group to consider this aspect of cancer control and treatment is clearly articulated – and is a topic that needs good, thoughtful science to buttress its standing in the overall field of oncology.

Response 1. The team thanks the reviewer for the supportive comments.

Comment 2. *In the absence of knowing the entire contents of the special issue, it is difficult to understand the ideal role and scope of this presumed lead article. Is it to serve as an Introduction or perhaps an Executive Summary? At present, it has elements of both. Like most working monographs, it would seem that an executive summary of the Halifax project would provide access to the curious, but time-limited readers who will not revel in the entire issue contents. This article makes some, inconsistent attempts to provide such a summary. Each of the hallmarks is reviewed in terms of their underlying biology, but only a few of these are complemented with summary conclusions of the review panels about priority agents or targets within a given hallmark. The elements of this section are very uneven. A synopsis view of what each review panel determined could be very useful. Perhaps these are included in a highlighted way in the individual articles/reports?*

Response 2. We appreciate the reviewers' pointing out the difficulty in understanding the scope of the article. This article is a capstone to the special issue, which contains a series of review articles on the cancer hallmarks. We have provided a new paragraph in the introduction that may help explain the status of the article, as follows (this is the same paragraph that was provided for Reviewer 1, question 2):

> This capstone paper describes the methods and results of a substantial effort by a large international group of biochemical and medical researchers, operating under the name of "The Halifax Project," sponsored by a non-profit organization, Getting To Know Cancer It summarizes and draws together material from a series of reviews on the hallmarks of cancer, presented in this special issue of Seminars in Cancer Biology, to present a conceptual framework for a new approach to cancer prevention and therapeutics. This approach involves the targeting of many high-priority anti-cancer mechanisms and pathways within a more comprehensive model of treatment and care. We refer to this as a "broad-spectrum" approach (i.e., an approach aimed at a broad spectrum of important mechanisms and pathways). The approach involves combinations of multiple low-toxicity agents that can collectively impact many pathways that are known to be important for the genesis and spread of cancer. By making extensive use of chemicals from plants and foods that have already been studied or utilized for cancer prevention and treatment, this approach offers a compelling rationale for addressing the underlying biology of cancer while being efficacious, non-toxic and cost-effective. We come together in the belief that a broad-spectrum approach of this type, in the context of a clinical environment including conventional treatment and attentive to optimal health, would provide real benefit for cancer patients. In this paper we describe the rationale for broad-spectrum therapeutics, detail

> the methods of the Halifax Project, summarize potential targets and agents related to eleven hallmark features of cancer, propose a research model for the development of broad-spectrum therapies, and call for action to advance this research model.

In addition, we also thoroughly revised the hallmarks section. The individual hallmark summaries are now, shorter, of more uniform length and level of detail, and in addition each states the priority targets and approaches selected by the hallmark teams.

Comment 3. *If the purpose is just to serve as an Introduction, then many aspects of the project process are well described. However, there is one critical element that is not clearly described. What kind of filter was placed on the quality of the studies considered by the working groups? This area of "complementary oncology" is littered with bad science. Indeed, this article nicely highlights many of the challenges of research with natural products as an example: sources, quality control, relevant doses, roles of matrices on biological effects versus reductionism, among many. Is greater value placed on in vivo experiments over in vitro ones, where there is a propensity to use exuberant concentrations? Table 2 in particular is only as good as the quality of the data that form the individual associations. Hopefully, the individual articles, if not this overview, provide a good sense of the data used to form conclusions. Otherwise, this exercise is unlikely to sway decision makers and practitioners in oncology. The IARC monographs serve as a useful approach to this type of evaluative process.*

Response 3. Thank you for pointing out this important point, which we agree was not adequately explained in the initial submission. We have composed a paragraph for the methods explaining our rationale for accepting a rather broad range of articles in support of the cross-validation effort. In addition, four paragraphs quoted below from the "Summary of findings on targets and approaches…" section comment further on the limitations of these data. An effort this comprehensive has not previously been attempted and the results therefore need to be regarded as a preliminary foray into this type of thought. The main signal for which we were searching was the existence of a large number of conflicting, contrary cross-hallmark interactions, which would indicate that the broad-spectrum approach was not feasible. We did not find this. The individual reviews provide further insight on the approaches, along with references for each of the cross-hallmark interactions.

Methods section (page 16 of clean copy):

> It is important to note that the cross-validation team was not given any restrictions for literature selection for this effort, and contributing authors were not restricted to cancer-related research. This approach was taken because it was realized at the outset that this breadth and specificity of knowledge does not yet exist in the literature. As a result, the types and sources of data gathered in this effort varied considerably, although original studies were consistently favored over review articles. Moreover, many studies that were cited in this effort considered only a chemical's ability to instigate or promote an action that mimics a hallmark phenotype in a manner directionally consistent with changes that have been associated with cancer. So while we refer to these as anticancer or tumor-stimulating, the specificity of these activities and their implications for cancer treatment cannot and should not be immediately inferred from this database. In other words, the tabular results from this aspect of the

project (Tables 1, 2 and 3) were only compiled to serve as a starting point for future research, rather than a conclusive guide to therapy.

Summary of findings on targets and approaches section (page 32):

There are a number of limitations that should be noted in this delineation of cross-hallmark relationships. First, the researchers who assembled these results were not asked to distinguish between direct effects on other hallmark areas and reported effects on other hallmark areas that may have resulted in an indirect or "bystander" effect mediated through a different mechanism. In many cases, but not all, this distinction was made. Therefore it is likely that some of the complementary interactions do not represent a fully independent cross-hallmark relationship, but rather are simply indicative of some sort of downstream effect (e.g., within a signaling cascade or via some other signaling molecule that exerts pleiotropic effects). However, we did not feel that this project needed to investigate the nature of these complementary interactions in detail. Instead, our main concern was focused on the possibility that a large number of cross-hallmark relationships might be revealed where actions with pro-carcinogenic or tumor-promoting potential had been reported. It was more important to identify contrary and controversial cross-hallmark interactions than complementary ones, since targets or approaches that exert pro-carcinogenic actions would normally need to be more carefully assessed (or avoided altogether) in the development of combination approaches or interventions.

The second limitation of these reports of cross-hallmark relationships is related to data quality. In some instances, the underlying evidence used to support the indication of a cross-hallmark relationships was robust, consisting of multiple studies involving detailed in vitro and in vivo findings. However, in other instances, the underlying evidence that was used to report the existence of a cross-hallmark relationship was quite weak (e.g., consisting of only a single in vitro study involving a single cell-type). Again, the overarching goal in this project was to create a foundation that would allow us to look systematically across the literature in each of these areas, to help us shape the selection of the targets and approaches. So although we realized that not all of these reports of cross-hallmark relationships represented the same level of evidence, we still wanted to examine available evidence to flag targets and approaches where pro-carcinogenic actions had been reported.

There was considerable debate within the task force over the value of tables containing only a simplified indication of a relationship (i.e., + or -) supported by evidence that varied considerably in quality. But since many individual studies and reviews that focus on therapeutic approaches fail to work systematically across the spectrum of incidental actions that might result from combining therapies, it was our opinion that a tabularized framework was the only way to ensure that we had assembled a complete view of cross-hallmark activity.

The types of approaches selected differed among different review teams. While some review teams selected all or mostly phytochemicals or plant extracts, some teams felt that the evidence for these was insufficient, and emphasized other types of molecules,

including drugs in development. These may pose more difficulties for translational investigators due to intellectual property, toxicity or other concerns, but may offer advantages in a more clear understanding of their mechanisms. We suggest, however, that the approaches as well as the targets presented in Tables 1 and 2 can be viewed as simply a model for broad-spectrum cancer therapies, rather than as a conclusive or final list. Some of the recommended approaches are clearly experimental, and further research will likely discover compounds, phytochemical or synthetic, that are not on this list that may be useful in a broad-spectrum approach.

Bioavailability of the phytochemicals chosen will also be a concern for future studies. However, the need for development of better preclinical models for screening compounds and testing rationally designed combinatorial therapies composed of compounds from any source is obvious, and should clearly be the first step in the development of the broad-spectrum approach.

Comment 4. *The authors likely underestimate the regulatory barriers to evaluating some of these interventions. The US FDA and institutional IRBs are clamping down on trials with supplements, despite the allowances in DSHEA, to require INDs for most studies. Combinatorial studies are even more difficult under current FDA guidelines. It would seem likely that other countries will be tightening their regulatory positions, as has China for example, rather than allowing them to remain more lax than US standards and approaches.*

Response 4. The section on regulations in different countries has been markedly shortened, due to considerations of space as well as complexity of the articles. The new paragraph discussing regulations is shown below, and acknowledges your point. We have considered the difficulty of work with the US FDA, however, and still feel that there is potential for work in the US, as explained further below.

Regulatory considerations (page 38):

> The United States has perhaps the most challenging regulations for drug approval, and regulations for mixtures are particularly complex. Some multicomponent formulas, have nevertheless been tested in clinical trials in the US [208,209], but are still being sold only as dietary supplements, without labeling for use in malignancy. The designation of the Botanical Drugs category may offer opportunities to broad-spectrum agents. A recent court decision declaring natural products unpatentable under US law adds an interesting wrinkle to the regulatory framework [210]. In Canada, development as a high-risk Natural Health Product could be considered [211]. China has a variety of regulatory categories that could be used for multicomponent natural product therapeutics [212]. The relevance of Chinese regulations for multi-targeted drugs has been explored [213]. In the European Union, the Marketing Authorization scheme for conventional drugs would need to be used, rather than the Traditional Herbal Regulation Scheme [214], increasing the challenge for developmental research. In India it is likely that New Chemical Entity approval would be required [215], since use in cancer would likely be considered beyond traditional herbal medicine usage. Japan allows herbal medicines to be registered as prescription or over-the-counter drugs [207]; prescription licensing appears likely for an anticancer therapeutic. A variety of regulations exist in other

countries, which are beyond the scope of this paper, and which would need to be explored individually. We expect that working under these strict regulations will be difficult, but we do not see it as impossible.

Response 4 continued: Regarding the difficulty of work with the US FDA, we would point out that natural product (biologics and botanicals) single agents or compounds are systematically evaluated using the same scientific rigor with which we evaluate other single agents and compounds for therapeutic use in cancer treatment. The current approach in evaluating the use of these natural agents is to use a multi-disciplinary science-based approach and the rigor with which we test other therapeutic agents. Several agents have been evaluated using this systematic approach informed by epidemiological, laboratory, preclinical prior to moving these promising agents to phase I-III clinical trials. These studies are funded by the NIH-NCI, Division of Cancer Prevention to evaluate safety, effectiveness and potential molecular targets and are further evaluated in phase I-II clinical trials. The NIH-NCI requires an FDA-IND prior to use in clinical trials. The FDA provides clear guidelines to investigators and the industry. Investigators are guided by the FDA staff through this process Several INDs have been issued to investigators evaluating these natural compounds with trials supported by the NIH and other agencies. INDs for the evaluation of multi-component compounds have also been issued. The work is difficult, but the FDA provides increasingly clear guidance for researchers interested in this area, which is readily available on their website.

Reviewer 4

Comment 1. *This paper describes a potentially important approach to cancer treatment. For those of us who have long felt that targeted therapy alone would probably not be adequate to achieve total remission of most cancers, the concept of using a "broad approach" with therapeutic agents in conjunction with natural compounds that elicit little or no toxicity and that have been shown to target multiple pathways in carcinogenesis seems entirely logical. The review teams from the Halifax Project have used a rational approach for the selection of targets for the broad approach therapy and the choice of specific natural compounds for these targets seems reasonable and supported by the literature. However, given the significant heterogeneity of individual cancers and the remarkable ability of cancer cells to resist treatment, this reviewer thinks that the proposed broad approach will enhance survival time and perhaps reduce cachexia, but will probably not lead to total remission of most tumors. Nevertheless, enhancement of survival time using relatively inexpensive approaches is an important goal and definitely worthy of evaluation.*

Response 1. We agree with the reviewer that the broad-spectrum agent is unlikely to cause curative remissions. This is why the situations we suggest for its use are not curative settings, but rather settings like early stage disease where aggressive treatment is not needed, hospice and palliative care and similar situations. Additionally, we address the concept directly in the following sentence at the end of the introductory section (page 12, clean copy):

> Because of continuous heterogeneity among cancer cells, and their propensity for genetic instability, even a broad-spectrum agent is unlikely to cause complete remission.

Comment 2. *This reviewer has only a couple of concerns that should be addressed. The authors have selected resveratrol, EGCG and curcumin as key natural compounds to be used for evaluation in conjunction with therapeutic agents. However, as the authors point out, these polyphenols are poorly absorbed. Moreover, NCI-supported chemoprevention trials with these agents have been only moderately successful or unsuccessful. Other compounds such as phenethyl isothiocyanate and silibinin have more favorable pharmacokinetics and would seem worthy of more attention than additional studies with agents that have limited bioavailability.*

Response 2. We do agree that silibinin (one of the selected approaches) and phenethyl isothiocyanate are interesting compounds, and have recognized them in the following sentences in the section Proposed Research Model (page 37 of clean copy):

> Some of the selected approaches, e.g. silibinin, appear to have favorable pharmacokinetics [194]. Other phytochemicals with favorable pharmacokinetics could also be considered for inclusion in a broad-spectrum agent, such as phenethyl isothiocyanate [195]

We are definitely aware of the problems with bioavailability of the polyphenols that have been most frequently selected by the hallmark review teams. However, several groups are working on methods to improve the bioavailable forms of these compounds, which is noted in the manuscript (page 38 of clean copy). One can examine in more detail, for example, curcumin. A few clinical trials have been initiated addressing the pharmacokinetics, safety, and efficacy of curcumin in chemoprevention trials targeting breast, colon, and pancreatic cancers. These trials found curcumin to be safe in specific formulations and available systemically outside the gastrointestinal tract with no dose-limiting toxicity. Based on these studies, it is clear that curcumin at doses ranging from 4-8 grams/day is bioavailable and bioactive mediated via anti-proliferative and anti-inflammatory effects. There has been some concern regarding bioavailability of curcumin which may be related to studies in the past that reported curcumin in plasma as a marker of bioavailability. However, more recent trials have examined and reported plasma PGE2 levels and PGE-M in urine and validated these to represent novel markers of bioavailability of curcumin in phase II clinical trials. Curcumin thus continues to be a promising agent. Several adjuvants (piperine, quercetin,), and formulations (nanoparticles and liposome encapsulated forms) are continuing to be developed to enhance bioavailability and bioactivity of curcumin.

Comment 3. *Another concern is that the authors have not included any discussion of food formulations that could well be useful for use along with therapeutic agents for cancer treatment. The authors are referred to studies with freeze-dried berry powder formulations which have been shown in animals to protectively modulate many of the targets selected by the Halifax Project and which have demonstrated efficacy against premalignant dysplastic lesions in the human oral cavity and esophagus and against rectal polyps in FAP patients. The authors are referred to the commentary by G.D. Stoner, Foodstuffs for cancer prevention: The preclinical and clinical development of berries, Cancer Prev Res 2:187, 2009 in which an approach to the standardization of berry powders and for the assessment of their preclinical and clinical efficacy is presented. Berry powders have been shown to exhibit antioxidant activity, inhibit phase I enzymes involved in carcinogen activation and stimulate phase II enzymes involved in carcinogen detoxification, reduce the proliferation of premalignant cells, and reduce inflammation and angiogenesis, stimulate apoptosis and cellular differentiation, promote cell to cell communication, and demethylate tumor suppressor genes associated with the Wnt signaling pathway in colon cancer presumably by their ability to down-regulate DNA methyltransferases (Wang et al Clin Cancer Res. 1:17, 2011; and more recent articles). Undoubtedly, other foodstuffs could have similar effects and may even*

work synergistically with berries if formulated in ways that concentrate and preserve their bioactive constituents.

Response 3. We certainly have to acknowledge the potential of food extracts. Although the hallmark review teams were not directed to examine foods, mainly because of concerns about bulkiness of therapeutic agents containing both food extracts and a number of other constituents, different types of dosage forms could certainly be considered that incorporate food extracts. Please note the added material in the following sentences from the Summary and conclusions section (page 43 of clean copy):

> And although this effort emphasized phytochemicals, it is also important and relevant to study defined botanical and food extracts. Standardized black raspberry extract, for instance, has produced positive results in human trials on apoptosis, angiogenesis and several specific targets selected in the project. [253]. Aged garlic extract [254] improved immunity in advanced cancer patients, and lyophilized strawberries [255] improved premalignant esophageal lesions.

Comment 4. *In summary, the authors are to be congratulated for taking on the enormous task of developing this broad based approach to the treatment of cancer and hopefully, it will lead to the allocation of significant funds to test the approach. This may well become a landmark paper in cancer research.*

Response 4. The team thanks the reviewer and shares the hopes expressed.

A Broad-Spectrum Integrative Design for Cancer Prevention and Therapy

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Abstract

Targeted therapies and the consequent adoption of “personalized” oncology have achieved notable successes in some cancers, however significant problems remain with this approach. Many targeted therapies are highly toxic, costs are extremely high, and most patients experience relapse after a few disease-free months. Relapses arise from genetic heterogeneity in tumors, which harbor therapy-resistant immortalized cells that have adopted alternate and compensatory pathways (i.e., pathways that are not reliant upon the same mechanisms as those which have been targeted). To address these limitations, an international task force of 177 scientists was assembled to explore the concept of a low-toxicity “broad-spectrum” therapeutic approach that could simultaneously target many key pathways and mechanisms. Using cancer hallmark phenotypes and the tumor microenvironment to account for the various aspects of relevant cancer biology, interdisciplinary teams reviewed each hallmark area and nominated a wide range of high-priority targets (83 in total) that could be modified to improve patient outcomes. For each target, a corresponding low-toxicity therapeutic approach was then suggested; many were phytochemicals. Proposed actions on each target and all of the approaches were further reviewed for known effects on other hallmark areas and the tumor microenvironment. Potential contrary or pro-carcinogenic effects were found for 3.5% of the relationships between targets and other hallmarks, and mixed evidence of complementary and contrary relationships was found for 7.8%. Approximately 67% of the relationships revealed potentially complementary effects, and the remainder had no known relationship. These results suggests that a broad-spectrum approach should be feasible from a safety standpoint. This novel approach has potential to help us address disease relapse, which is a substantial and longstanding problem, so a proposed agenda for future research is offered.

Keywords:

Multi-targeted, cancer hallmarks, phytochemicals, targeted therapy, integrative medicine

Introduction

Cancer is a source of significant and growing mortality worldwide, with an increase to 19.3 million new cancer cases per year projected for 2025. More than half of cancer cases and mortality occur in low- and middle-income countries, and these proportions are expected to increase by 2025 [1]. Current treatments for cancer include surgery, radiotherapy and systemic treatments comprising cytotoxic chemotherapy, hormonal therapy, immunotherapy, and targeted therapies [2]. Cancer continues to stymie clinical treatment efforts, however, and the search for effective therapies continues.

This capstone paper describes the methods and results of a substantial effort by a large international group of biochemical and medical researchers, operating under the name of "The Halifax Project," sponsored by a non-profit organization, Getting To Know Cancer. ~~to develop~~ It summarizes and draws together material from a series of reviews on the hallmarks of cancer, presented in this special issue of Seminars in Cancer Biology, to present a conceptual framework for a new approach to cancer prevention and therapeutics. This approach involves the targeting of many high-priority anti-cancer mechanisms and pathways within a more comprehensive model of treatment and care. We refer to this as a "broad-spectrum" approach (i.e., an approach aimed at a broad spectrum of important mechanisms and pathways). The approach involves combinations of multiple low-toxicity agents that can collectively impact many pathways that are known to be important for ~~the immortalization of cancer cells and the~~ genesis and spread of cancer. By making extensive use of chemicals from plants and foods that have already been studied or utilized for cancer prevention and treatment, ~~it is our contention that~~ this approach offers a compelling rationale for addressing the underlying biology of cancer while being efficacious, non-toxic and cost-effective. ~~As a large international group of researchers,~~ We come together in the belief that a broad-spectrum approach of this type, in the context of a clinical environment including conventional treatment and attentive to optimal health, would provide real benefit for cancer patients. In this paper we describe the rationale for broad-spectrum therapeutics, detail the methods of the Halifax Project, summarize potential targets and agents related to eleven hallmark features of cancer, propose a research model for the development of broad-spectrum therapies, and call for action to advance this research model.

1. Rationale for Broad-Spectrum Approach

Primary motivations for the development of a broad-spectrum approach stem from the distinct limitations that are evident in many current targeted therapies and the personalized medicine paradigm ~~despite some clear successes~~. Molecular target therapies represent a significant advance in the treatment of cancer. They include drugs such as imatinib, an Abl tyrosine kinase inhibitor that has made chronic myelogenous leukemia a more manageable disease, and inhibitors of vascular endothelial growth factor receptor (VEGFR), such as sunitinib, sorafenib and bevacizumab, ~~that are now standard of care~~ used in renal and colon cancers [2]. Other important treatments based on tumor-specific targets are now in use, including examples such as epidermal growth factor receptor (EGFR) inhibitors (gefitinib, erlotinib) used in lung cancer,

and the Her2 inhibitor trastuzumab used in breast cancer ~~and the Braf inhibitor ipilimumab used in melanoma~~. Another approach is the synthetic lethal model [3]exemplified by research on poly ADP ribose polymerase (PARP) inhibition, in which mutational loss of one or more redundant components of a cell survival pathway in tumorigenic cells confers selective sensitivity to drugs that target remaining pathway components.

These drugs target cells bearing one, or at most a few mutated gene products or other abnormalities not found on normal cells. In the therapeutic context, the action of the targeted agents can efficiently address malignant cells, without some of the effects on normal cells notorious in cytotoxic chemotherapy. This enables therapeutic responses and remissions. Over time, however, the genetic heterogeneity of ~~primary (and later metastatic)~~ tumors increases, ~~giving rise to the potential for~~ engendering resistance to treatment. ~~As~~ Resistant cells ~~survive this pressure,~~ drive the emergence of increasingly aggressive disease ~~is seen,~~ through clonal expansion and clonal evolution [Figure 1]. ~~To further complicate matters, the evolved tumors may have altered~~ Epigenetic modifications may also alter patterns of gene expression ~~from epigenetic modifications as well, and can lead to disease relapses.~~ ~~These~~ Relapses may occur after only a few months, and tumors reappear, sometimes in exactly the same areas in which they originated ~~often resistant to previous therapy~~ [4]. Moreover, targeted agents are not without serious side effects, such as treatment-related mortality with bevacizumab and cardiopulmonary arrest with cetuximab. Meta-analysis of trials of recently approved cancer drugs including targeted therapies versus older drugs showed increased rates of grades 3 and 4 toxicity (OR=1.52), treatment discontinuation (OR=1.33) and toxic deaths (OR = 1.40) [5].

[Figure 1 about here]

The efficacy shown to date with targeted therapies, aside from now-established treatments such as bevacizumab and trastuzumab, is nevertheless still limited. Sunitinib, for instance, extends overall survival by 4.6 months in renal cancer, compared with the previous treatment of interferon-α [6]. While statistically significant, this degree of improvement is small comfort to afflicted patients, and challenges the extraordinary monetary investment in drug development as well as costs to the medical system that targeted therapies represent. The MOSCATO 01 trial of molecular triage was able to treat 25 of 111 patients with a variety of advanced cancers using therapies targeted to genomic alterations assessed from tumor biopsies [7]. Of these, 5 patients (20%) experienced partial response and 56% had stable disease. Based on the entire population of 111 patients, this is a partial response of less than 5%, suggesting limited efficacy to date, an outcome also seen in some other studies ~~. A pilot study of 11 patients using whole genome sequencing and whole transcriptome sequencing was able to treat one patient with an actionable target, who had a short-lived PET/CT response and a dramatic reduction in pain, representing overall efficacy that is still quite limited~~ [8]. On a more hopeful note however, a combination of pertuzumab with trastuzumab and the chemotherapy agent docetaxel was recently found to extend overall survival among the subset of breast cancer patients whose tumors express Her-2 by 15.7 months [9].

Interestingly, harnessing the body´s immune response against the tumor can also result in impressive durable clinical responses, perhaps because the immune system is a paragon of adaptability and can deal with changes in the mutational landscape of cancer to prevent escape from the therapeutic effect. The immunomodulatory antibodies now in advanced clinical trials include ipilimumab (already licensed) as well as nivolimumab and pembrolizumab (licensing anticipated soon) neutralizing two different inhibitory pathways that block anti-tumor T cell responses. These agents have achieved some successes in treating late stage cancers refractory to essentially any other treatments [10]. But even with these agents, response rates are still low and predicting who will respond is a challenge [11,12].

Many of these therapies are somewhat narrowly described as “personalized” because patients’ tumors must be tested for specific mutations to stratify patients to the correct therapy. Viewed in the larger context of individual biological variation, of course, specific mutations drive only the smallest degree of personalization. Truly personalized treatment approaches can be seen to include a much more comprehensive assessment of genetic and even lifestyle factors, such as nutritional, biobehavioral, and exercise habits, along with other host variables such as inflammation and immune status. Such an approach to personalizing treatment can be found in the practice of integrative medicine, which played a significant role in the initial development of this model of broad-spectrum cancer therapy. Some definitions of integrative medicine stress simply the inclusion of complementary and alternative therapies alongside orthodox treatment [13]. A more relevant definition emphasizes a ~~comprehensive,~~ multi-intervention treatment paradigm that utilizes diet, mind-body and physical activity therapies in addition to conventional therapies and dietary supplements [14], based on laboratory testing that enables comprehensive personalization. ~~Systematic laboratory testing for cancer-promoting states such as inflammation and oxidative stress, or for cancer-related mutations allows lifestyle and supplementation recommendations to be personalized. Clinical experience with personalization of this type has revealed the notable range of individual variation in genetic and biochemical characteristics found in cancer patient populations, making a compelling case for broad-spectrum therapies [14].~~

The stratification of patients for these targeted and personalized therapies poses practical challenges. As indicated earlier, over 50% of the increase in cancer incidence by 2025 is projected to occur in the developing world [1]. As industrialization develops in lower-income countries, occupational cancers are expected to increase, potentially aggravating this situation [15]. ~~Childhood cancers may also be linked to occupational exposures including solvents, paints and automotive-related products~~ Cancer treatment in many of these countries is already becoming a social-economic challenge due to the expense and medical infrastructure required [16], and the new generation of treatments may further strain local resources. Currently, the platforms used for testing to personalize regimens include whole exome or whole genome sequencing, whole transcriptome sequencing, and comparative genomic hybridization~~, RNA sequencing, gene expression analysis, and other methods. Metabolomic analyses and small gene sets are~~ with still others in development. It is likely that such tests, and related expense, will proliferate in the future~~, following the recent progress in genetic analysis~~. Managing

treatment toxicity is also a taxing and complex problem, as these toxicities necessitate additional medical interventions.

~~From a cost standpoint~~, The expense of the new targeted therapies ~~at present~~ is also concerning. Eleven of twelve drugs approved by the US Food and Drug Administration in 2012 were priced above $100,000 US per year per patient – perhaps not surprisingly in view of the accelerating costs of drug development [17]. Clinicians have drawn attention to these high costs: in 2013 more than 100 experts in chronic myeloid leukemia coauthored a paper calling for lower prices and broader access to these drugs [18]. The excessive costs have resulted in drugs not being approved for use by national or regional governments where cost-benefit analyses figure in approval processes [19]. While costs are expected to decrease after expiration of patents on the drugs, the costs for treatment in low- or middle-income countries may continue to be problematic. ~~Adding to the expense and logistical challenges is the required use of genetic testing for stratification of patients in both clinical trials and therapeutic application.~~ The potential for unsupportable financial stress on health systems challenges the research community to explore other treatment models that can be more sustainable in the face of the ~~accelerating~~ worldwide increase in cancer incidence.

The broad-spectrum approach that we describe here is primarily intended to address the two major issues of therapeutic resistance and cost, ~~and not to remedy all of the shortcomings of conventional cancer therapy~~. It is based on many of the insights of genomic sequencing in cancers. We now know that cancers harbor significant genetic heterogeneity, even within a single patient [4]. ~~Patterns of relapse following targeted therapies clearly demonstrate that the targeted therapy approach, despite obvious successes, cannot completely manage cancer.~~ Based on this heterogeneity, cancers routinely evolve resistance to treatment through switching from one growth pathway to another, ~~based on this genetic heterogeneity~~. The proposed strategy employs the basic principles of rational drug design, but aims to stem cancer growth by precisely targeting many ~~disease-specific~~ growth pathways simultaneously. ~~It should be noted that efforts are now being made to combine molecular targeted therapies and other drugs so that more than one target can be inhibited. Although lack of therapeutic success and significant toxicity and costs when using more than one agent have made it challenging to treat with multi-drug combinations of current agents, some progress is being made.~~ Some progress is now being made in combining targeted agents so that more than one pathway can be affected, but lack of therapeutic success and significant toxicity and costs make this a challenge [20-23].

We see the broad-spectrum approach as one that is complementary to existing therapies, preferably within the context of a genuinely integrative clinical system. Clinical situations in which such an approach might prove useful include (a) as a follow-up to conventional adjuvant treatment; (b) in situations of rare cancers and disease stages for which no accepted treatments exist; (c) for patients who do not tolerate conventional chemotherapy, hormonal therapy or targeted therapies; (d) for patients who experience relapse or progression after targeted treatment; (e) in hospice or palliative care patients where low- or non-invasive strategies are a legitimate and humane option; and (f) in situations in which high-cost agents

cannot be obtained. Because of continuous heterogeneity among cancer cells, and their propensity for genetic instability, even a broad-spectrum approach is unlikely to cause complete remission. However, the design of this approach posed a substantial theoretical challenge, for which we chose to use the hallmarks of cancer as a broad organizing framework.

1.1 Hallmarks of cancer

Douglas Hanahan and Robert A. Weinberg first published their concept of the hallmarks of cancer in 2000 [24]. The hallmarks "constitute an organizing principle that provides a logical framework for understanding the remarkable diversity of neoplastic diseases." This framework encompasses the biological capabilities that cells acquire during the development of cancers that allow them to become malignancies as we know them. Six hallmarks were proposed in the 2000 publication: sustained proliferative signaling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis and resisting cell death. The concept of the hallmarks became widely recognized and influential. In 2011, Hanahan and Weinberg expanded on the initial hallmarks to include other areas of cancer biology that they felt were equally important [25]. They pointed out two enabling characteristics critical to the ability of cells to acquire the six hallmarks, and two new hallmark capabilities. They also singled out the crucial nature of the complex tumor microenvironment in the appearance of the cancer phenotype. The enabling characteristics are genomic instability and tumor-promoting inflammation; the new hallmarks are deregulating cellular energetics and avoiding immune destruction.

~~In general,~~ The hallmarks framework ~~is useful because it~~ helps to define ~~the~~ domains in which high priority targets can be identified for therapeutic targeting. Hanahan and Weinberg point out that agents are in development that target each of the hallmarks. They also note, however, that in response to targeted therapy, cancers may reduce their reliance on a particular hallmark capability, such as angiogenesis, and instead heighten the activity of another capability, such as invasion and metastasis [26]. This reaction has been clinically verified in the case of glioblastoma [27].

Another model, which was proposed by Vogelstein et al. in 2013 [4], also attempts to describe the mechanisms and pathways that are relevant to many cancers. In this model, 12 major signaling pathways that drive cancer growth have been elucidated, including signal transducers and activators of transcription (STAT), NOTCH, DNA damage control and 9 others. These pathways are classified into three cellular processes underlying tumor growth: cell survival, cell fate and genome maintenance. Individual patients with the same cancer can have mutations on different pathways, leading to inter-patient heterogeneity. Yet within each patient there is also substantial heterogeneity, ~~Heterogeneity exists between patients with different driver mutations, and~~ both within each patient's primary tumor, ~~but also~~ and among and within metastases, with significance for treatment strategies. For instance, the smallest metastases visible through medical imaging may already have thousands of cells that harbor mutations rendering them resistant to current drugs [28].

Cancer mutations, moreover, are not simply a series of isolated targets. Beneath the surface of the cancer genome is a notably complex cellular signaling network, filled with redundancies. The elucidation of rational therapeutic combinations requires dynamic mechanistic models that reach beyond simple targeting [29]. What propels growth, dissemination and thus ineffective treatment and drug resistance actually appears not to be pathways acting in isolation but interconnected, multidirectional and dynamic networks [30]. Even sorafenib, which inhibits multiple kinases, is susceptible to the rapid development of resistance deriving from crosstalk in pathways such as phosphatidylinositide 3-kinase (PI3K)/Akt and Janus kinase (JAK)-STAT, hypoxia-induced signaling or the epithelial-mesenchymal transition [31]. Conventional drug discovery programs are now contemplating systems biology approaches aimed at furthering the network approach to pharmacology. The interdependence of cytokines, chemokines, growth factors, transcription factors, and their resulting proteomes, together with their relevance to cancer prevention and treatment [32], makes systems biology approaches most attractive [33]. This realization makes the significance of a broad-spectrum approach to cancer of even greater importance.

Pharmacologists are not alone in their recognition of the heterogeneity of cancer. A least one clinical center recognizes the significance of this heterogeneity, and intervenes with broad-spectrum approaches to respond to it. In a 2009 book, Life Over Cancer, based on a clinic in operation since 1980, K.I. Block lays out a model of nutraceutical-based targeting of nine "pathways of progression" and six metabolic factors impacting the challenges faced by all cancer patients [34]. The nine growth pathways are proliferation, apoptosis, treatment resistance, immune evasion, angiogenesis, metastasis, cell-to-cell communication, differentiation and immortality. Multiple targeting of these pathways with natural products ~~such as green tea, curcumin and reishi mushrooms is presented as a means of~~ is used to simultaneous address multiple interconnected growth pathways. Molecular profiling ~~is used to~~ maps the growth pathways of the individual patient and suggests relevant natural product intervention. The six metabolic "terrain factors" are oxidation, inflammation, glycemia, blood coagulation, immunity and stress chemistry. ~~Standard laboratory tests are used to assess the terrain factors, each of which is acknowledged to depend on a multiplicity of processes. Terrain factors also mold the environment surrounding the tumor, and affect treatment response and quality of life.~~ Terrain-focused interventions are tailored to patients' laboratory test results ~~as well as disease type, symptoms and treatment side effects~~. Interventions include elimination of maladaptive lifestyle patterns ~~(e.g. smoking, alcohol, sleep deficits)~~, adjusting exercise habits, improving diet ~~to optimize metabolic processes affecting the terrain~~, implementing biobehavioral strategies to diminish adverse consequences of unabated stress/distress, and using natural products and medications that affect specific targets ~~for terrain variables~~ such as C-reactive protein (CRP) [35], interleukin-6 (IL-6), nuclear factor κ-beta (NF-κB) [36], prostaglandin E2 (PGE2) and leukotriene B4 (LTB4)[37]for inflammation. Clinical observations and ~~further~~ literature review suggest potential ~~outcome-related~~ efficacy for this system in breast cancer (including a near-doubling of survival time of breast cancer patients in integrative care) and potentially other cancers [38,39]. Essentially, Block's clinical model systematically addresses multiple targets and pathways ~~and primary as well as compensatory and alternative pathways by profiling each patient -- both tumor and microenvironment --~~ through a specific

and selective broad-spectrum approach to treatment. While this system was developed in clinical practice, quite independently from the discussion of hallmarks and enabling characteristics by Hanahan and Weinberg, the conceptual overlap is obvious. That these concepts have already been used in clinical treatment provides powerful support for the viability of a carefully designed broad-spectrum approach.

The model we propose to use to develop a sound framework for a broad-spectrum approach recognizes these broad areas of conceptual overlap and agreement, and can be considered to best align with the hallmarks of cancer framework [24]. ~~Our framework encompasses the molecular and metabolic diversity of malignancy recognized in Hanahan and Weinberg's hallmarks, Vogelstein's 12 growth pathways, Block's pathways of progression and terrain factors (15 in total), and other emerging research.~~ For the purposes of this project, we treat the 6 hallmarks, 2 enabling characteristics, 2 emerging hallmarks, and the tumor microenvironment equally as hallmarks of malignancy. From a design standpoint, each of these individual areas encompasses an important aspect of cancer's biology, so each was seen as important to consider for a therapeutic approach aimed at a wide range of high priority targets.

In mid-2012, the framework for this project and approach were shared with Douglas Hanahan. He later independently provided support for this type of approach in a paper, "Rethinking the war on cancer" [40]. Using a military metaphor, he suggests a three-dimensional cancer "battlespace" plan that attacks cancer in a full-scale war rather than individually targeted skirmishes. The first dimension is disruption of cancer's many capabilities, specifically those figuring in the hallmarks. Rather than just removing one capability, as targeted therapies do, he explains that an ideal approach should target all the hallmark capabilities. The second dimension ~~defined by Hanahan~~ is defense against cancer's armed forces, implying specific targeting of the accessory cell types in the tumor microenvironment, such as tumor-promoting inflammatory cells. The third dimension ~~that he describes~~ represents the multiple battlefields of cancer: primary tumor, tumor microenvironment, lymph and blood vessels through which tumors disseminate, draining lymph nodes and distant organs. This dimension suggests still more targets.

A rapidly developing sub-discipline in oncology is the application of genetic and immune analysis of tumor tissue and the concomitant use of personalized therapies and prescriptions. These analyses~~, many of them containing multiple genes,~~ allow better stratification of patients to treatments and clinical decision-making [41]. In the case of breast cancer alone, tests range from Her-2 testing, the basis of trastuzumab treatment, through Oncotype DX®, a 21-gene panel, to the Symphony™ suite of tests by Agendia which analyzes dozens of genes. These complex analyses assist in treatment decisions based on correlations with clinical outcomes ~~(not, e.g., driver genes)~~ by predicting treatment response, risk of recurrence and outcome. They suggest the size of the network of genes that affect just one cancer, and emphasize the significance of a broad-spectrum attack. ~~A pilot study using personalization of targeted therapies based on analyses of a multiple-tumor marker panel in pediatric brain tumor patients provides a positive example of the use of such panels~~ Clinical utility of these tests is still under review [42]. ~~Using morphoproteomic data, a panel of 8 tumor markers, and records of patient~~

~~histories, the investigators were able to obtain 1 complete response and 2 continuous complete remissions in 8 patients.~~

The fact remains that, despite impressive progress in genomic and gene expression profiling, it is often impossible to fully characterize the range of immortalized cell variants within any given cancer. The perspectives offered by Hanahan and Block, as well as by the recognition of the network aspects of signaling pathways, however, suggest a larger number of targets may need to be reached. So the 138 driver genes, together with the 12 signaling pathways that comprise them, in addition to the molecular contributors to the hallmarks, and Block's nine pathways of progression and six terrain factors, help us delineate some of the most significant targets that should be taken into account in development of a broad-spectrum approach.

2. Methods

The effort to develop the concept of broad-spectrum targeting of cancer through a complex combination of agents, emphasizing naturally occurring chemicals, was developed by a non-profit organization, Getting To Know Cancer, and implemented within an initiative called "The Halifax Project." The aim of the project was to produce a series of reviews of the cancer hallmarks that could collectively assess and prioritize the many target choices that exist, and also identify non-toxic chemicals (primarily from plants or foods) that could safely be combined to produce an optimized broad-spectrum solution that has both prophylactic and therapeutic potential. To that end, it was envisioned that eleven teams of researchers would produce reviews on the ten cancer hallmarks plus the tumor microenvironment, which was treated as a hallmark for the purposes of this project ~~to signify its importance~~. Each review was to describe the hallmark, its systemic and cellular dysfunctions, and its relationships to other hallmarks. A priority list of relevant therapeutic targets and corresponding approaches suited to those targets was requested, along with a discussion of research needed in the context of goals of the project. Natural compounds were emphasized because of the growing body of literature that supports the low toxicity and interesting potential that many of these substances have demonstrated (i.e., as targeted therapeutics or in cancer prevention), while recognizing the variable effectiveness of these compounds in human trials as well as the undocumented safety or frank toxicity concerns with many natural products [43].

In recognition of the network of signaling pathways involved not only in drug resistance but the interconnection and maintenance of all the hallmarks, the project implemented a cross-validation step in the evaluation of targets and approaches. Because of the diversity of the targets involved in the 11 hallmark areas, it is not unreasonable to suspect that inhibiting or stimulating a target relevant to one hallmark may have an adverse growth effect or clinically adverse effect on a target in another hallmark. For instance, reducing DNA damage is a potential target for counteracting genomic instability. Activation of the immune system can counter DNA damage by eliminating damaged cells. However, activation of the immune system, while reducing overall levels of DNA damage, can contribute to chronic inflammation. [44].

Similar considerations apply to therapeutic approaches. For instance, triptolide, a component of the Chinese herb *Tripterygium wilfordii*, is known to cause apoptosis in cancer cells [45]. Extracts of the herb have been used in clinical trials for a variety of inflammatory and immune-linked conditions, and have demonstrated both anti-inflammatory and immune suppressant activity, raising concern for its effect on immune evasion [46,47].

To address this issue, a specially designated cross-validation team was created within the project to evaluate all selected targets and approaches, i.e., to determine whether the inhibition or activation of targets, and the application of approaches, would have negative effects on other hallmarks. Each potential target-hallmark or approach-hallmark interaction was assessed to determine whether the pair had a complementary interaction (i.e., the interaction of the target or approach with the hallmark facilitated anticancer activity), a contrary interaction (i.e., the interaction of the target or approach with the hallmark had a potential adverse tumor-stimulating or tumor-progression effect), a controversial interaction (i.e., mixed indications of anticancer and tumor-stimulating effects), or no known relationship.

It is important to note that the cross-validation team was not given any restrictions for literature selection for this effort, and contributing authors were not restricted to cancer-related research. This approach was taken because it was realized at the outset that this breadth and specificity of knowledge does not yet exist in the literature. As a result, the types and sources of data gathered in this effort varied considerably, although original studies were consistently favored over review articles. Moreover, many studies that were cited in this effort considered only a chemical's ability to instigate or promote an action that mimics a hallmark phenotype in a manner directionally consistent with changes that have been associated with cancer. So while we refer to these as anticancer or tumor-stimulating, the specificity of these activities and their implications for cancer treatment cannot and should not be immediately inferred from this database. In other words, the tabular results from this aspect of the project (Tables 1, 2 and 3) were only compiled to serve as a starting point for future research, rather than a conclusive guide to therapy.

~~Conceptually speaking,~~ Targets or approaches that have a substantial number of "contrary" assessments are less attractive for inclusion in the broad-spectrum approach. On the other hand, the use of targets and approaches that appear to have the potential for multiple complementary interactions is consistent with principles of rational drug design, and akin to efforts to design "dirty" drugs (a pharmacological term for drugs with multiple targets – as opposed to single targets -- aimed at multidimensional conditions) [48]. Further evaluation of such "dirty" targets and approaches could be undertaken through more specific application of network pharmacology, for which new tools are currently becoming available [49]. The tabulated results, which appear in the individual reviews, are discussed in a later section of this paper.

The review teams needed for the Halifax Project were formed by first circulating an email to a large number of cancer researchers, seeking expressions of their interest in participation. The email was circulated in July 2012 by Getting To Know Cancer, and scientists were encouraged to

submit their details on a dedicated webpage that offered additional project detail. From the pool of 703 cancer scientists who responded to the email, 11 team leaders were selected to each lead a group in producing a review of each hallmark, and an additional leader selected for the cross-validation team. Those leaders were then asked to form their own teams (by drawing from the pool of researchers who expressed interest in the project, and from their own circles of collaborators). Ultimately, 12 teams were formed. Team members were each encouraged to engage a junior researcher as well. This led to fairly large teams but it allowed us to distribute the effort considerably. Team leaders all received project participation guidelines; extensive and ongoing communication from the project leader, Leroy Lowe; copies of the relevant papers of Hanahan and Weinberg; and copies of Life Over Cancer by Block [34] as an example of practical clinical implementation of the broad-spectrum approach. In addition to the two teams, the two guest editors, Anupam Bishayee and Keith Block, were selected for this special issue of *Seminars in Cancer Biology* in which the team reviews are published.

The team leaders and other team members who were able to attend the project workshop met in Halifax, Nova Scotia in August 2013 to discuss the project. Drafts of hallmark team papers were submitted in advance, and summary presentations made at the meeting. Other subject matter presentations included presentations on research funding in the natural products area (Jeffrey D. White, Office of Cancer Complementary and Alternative Medicine, National Cancer Institute) and the concept of driver and passenger genes (Bert Vogelstein, Johns Hopkins). Presentations on integrative cancer therapeutics made at the meeting are summarized below (Keith Block, Penny Block, Block Center for Integrative Cancer Treatment). Group discussions were held to facilitate communication among teams and project staff, and to assist teams in exploring the requirements and rationale for selection of targets and approaches.

Each hallmark team contained the following specialists: a lead author with demonstrated expertise in the hallmark area; domain experts who produced the descriptive review; anticancer phytochemical specialists; oncologists; and support researchers. The cross-validation team conducted background literature searches on the submitted targets and compounds from each review team, verifying their activity in relation to the other hallmarks. This team assessed tradeoffs through determining whether activities of one set of targets and compounds had effects that were complementary to, contrary to, or neutral towards the anticancer activities of each of the other topic areas. Results of the cross-validation effort were tabulated and reviewed by the individual teams. Ambiguous results and areas of disagreement were reconciled, and the tables were ultimately incorporated into each hallmark review.

2.1 Selection of targets and approaches

It was assumed from the outset that, in a translational project aimed at the development of a broad-spectrum approach, there would be a practical upper limit to the number of potential targets in any given cancer that could be targeted. So each hallmark team was asked to select and prioritize up to 10 relevant targets for their hallmark area (bearing in mind that each target would serve as a starting point for the identification of a suitable low-toxicity approach that might be used to reach that target). In theory, it was understood that this could lead to as

many as 110 targets for the entire project, and since the teams were also asked to select one therapeutic approach for each target, a maximum of 110 potential therapeutic approaches that may need to be combined.

An "approach" was defined in this project as (1) a technique that will cause the body to respond in a manner that will act on the target (e.g., fasting, exercise etc.), or (2) a procedure involving an entity that can act on the target (e.g., phytochemical, dietary modification, synthetic drug, vaccination with peptides, locally administered oncolytic virus etc). Teams were then asked to identify "favored" approaches with patient safety as a top priority (i.e., least likely to cause harm or side effects even in combination with many other approaches). In addition to safety, other practical considerations for choosing favored approaches were suggested as follows:

- **Efficacy** – Greatest potential to achieve the desired action on the intended target across the widest possible range of cancer types
- **Cost** – Less expensive is better, and by no means cost prohibitive
- **Intellectual Property** – Free of intellectual property constraints if at all possible. Approaches that do not have patents, that cannot be patented, and/or those that have patents that are expired are to be given priority over those that have existing patents.

2.2 Target selection

~~During the preparation of the hallmark reviews, and during the group meeting in August 2013,~~ Extensive discussion took place about the principles of target selection in the context of a broad-spectrum therapeutic approach. Certainly targets that are unique to cancer cells and tumor microenvironments, and that are not known to cause side effects when inhibited pharmacologically, would be a primary consideration. ~~Other sources of disruption, such as that~~ Targets induced by viruses or known carcinogens, would also be major considerations. Consideration of the nature of mutations in the cancer genome and the role of epigenetic modification were also discussed.

It is understood that great effort has been made to sequence the cancer genome to identify the most common mutations seen in different cancers. It is also known that different driver mutations may give rise to variant tumor cells, and the number of driver mutations required is limited, with just 2-8 per patient, which could potentially be assessed through whole genome sequencing of individual cancer patients. However, questions arise about treatment, since most of the currently available drugs are not potent enough to target all susceptible cells. Moreover, the toxicity of existing drugs, if administered in combination protocols, is severely limiting, even at the reduced dosages that may be possible when using multiple agents. ~~Therefore it was generally agreed that there is~~ A strong rationale supports focusing on low toxicity chemistry (e.g., such as that which has been demonstrated by many anticancer and chemopreventive phytochemicals ~~that have been reported in the peer-reviewed literature~~) as the foundation for a broad-spectrum approach. ~~It was also noted that~~ A number of phytochemicals ~~have also been demonstrated to~~ enhance absorption of other natural products through such mechanisms as cytochrome P450 modification [50], which could also enhance the

possibilities for low-toxicity treatment, i.e., by reducing dosages needed for effective treatment.

~~An additional consideration that was discussed is that~~ Many driver genes are actually tumor suppressor genes, and in these cases, it is the loss of the tumor suppressor gene that allows development of cancer. Drugs cannot target these missing genes. Rather they must target unopposed pathways, such as pathways that are active upstream from the missing suppressor gene. For instance, the tumor suppressor forkhead box 0 (FOX0) normally causes apoptosis. If FOX0 is inactivated in cancer, an unopposed pathway upstream from it is the PI3K/Akt1 signaling pathway, which could alternatively be targeted [51]. The MAP/ERK/MEK pathway, however, can act as a substitute or compensatory pathway to PI3K/Akt1. So, in order to effectively shut down replication, it would seem necessary to address these targets as well.

~~It was further recognized that~~ Cancer-related signaling pathways, including even those that become driver pathways, are also epigenetically modified prior to their genetic modification in cancer pathogenesis [52]. This suggests an emphasis on chemoprevention or treatment of very early cancers. Targeting may be more straightforward to achieve under these conditions, since it is easier to modulate wild-type pathways pharmacologically than to treat the consequences of the onset of widespread aneuploidy. In this case, the cancer phenotype may well precede the cancer genotype by years or more. Combining knowledge of genetic and epigenetic changes in a particular tumor may result in the targeting of key pathways with fewer agents and reduced cost.

A more general consideration is that both direct and indirect targets and approaches can be considered. Direct targets are those that are familiar to us from targeted therapies – oncogenes, tumor suppressor genes, signaling pathways. Indirect approaches, however, are also potentially useful. For instance, evasion of the immune system is a hallmark of cancer [24], and immunomodulatory targets and approaches are appropriate to support the capacities of immune cells to eliminate tumor cells. Immune regulators are, in a sense, inherently multi-targeted due to the complexity of the responses they induce [53]. However, immunity is frequently compromised in patients under treatment with cytotoxic chemotherapies, as well as in the post-surgical period. Consideration of immune system approaches that also support the capacity of patients to tolerate or recover from surgery or toxic therapies ~~would provide an~~ indirectly supports the health of cancer patients [54]. The potency of the immune system is illustrated by findings that chemotherapy may enhance anti-tumor immunity if given in the correct sequence, and that cancer refractory to chemotherapy or immune modulation alone may become susceptible to both together [55].

2.3 Approach selection

The need for low-toxicity agents as constituents suggested that phytochemicals –especially those “pre-screened” in humans owing to their presence in foods or traditional medicines -- should be carefully considered during approach selection. Each hallmark team therefore included cancer researchers who had considerable experience working with phytochemicals.

~~The selection of approaches was ultimately guided by the targets selected by each team. However, general principles of selecting approaches were discussed at the Halifax meeting.~~ In considering phytochemicals and other low-toxicity agents for inclusion in a broad-spectrum approach, however, several limitations in the literature promptly become clear.

First, the level of evidence for the effects of natural products on particular hallmark targets varies widely. The status of laboratory studies and clinical trials on several well-known phytochemicals, e.g. resveratrol, epigallocatechin gallate (EGCG), curcumin, lycopene and others, was recently reviewed [56]. The pleiotropic nature of the effects of these agents on apoptosis and arrest of cell growth has been emphasized, and their potential use in association with chemotherapy drugs has been acknowledged. Novel strategies based on a strategic combination of phytochemicals with broad-spectrum action together with radiation or chemotherapy agents aimed at overcoming resistance to apoptosis and enhancing sensitivity to treatment are also currently being considered [57,58].

Second, considerable clinical experience with combinations of phytochemicals and other natural agents in treatment of cancer patients exists~~, and has been discussed elsewhere [36]~~. Detailed knowledge of the pharmacological effects of combinations of phytochemicals, however, is limited.~~, both in clinical trials and in laboratory animal models. It should be noted that~~ There is a large literature on herbal combinations used in traditional Chinese medicine in both the laboratory and clinic [59-61], but the quality of ~~previously conducted~~ older clinical trials is generally low. Additionally, laboratory studies of herbal medicines often use concentrations far higher than are clinically achievable. Supra-physiological concentrations can produce artefactual or irrelevant mechanisms of action or cause toxicity. The limited bioavailability of major phytochemicals makes this especially concerning, although products with improved bioavailability are in development [62]. In general, phytochemical research ~~needs to become more mainstream, and~~ merits rigorous attention if we hope to gain a more detailed understanding of how these compounds affect the cancer hallmarks. Basic research needs to be followed up with better-designed and statistically-powered clinical trials, if we hope to fully realize the therapeutic potential of phytochemicals.

In addition to laboratory studies and clinical trials, approaches may be suggested by epidemiological studies and the observations of integrative medicine, which uses diet and lifestyle therapies to affect medical conditions including cancer. Observational studies of soy consumption, along with corroborating evidence from clinical studies, suggest that dietary consumption of soy foods consistent with levels in the Japanese diet (2-3 servings daily, containing 25-50mg isoflavones) may be associated with reduced risk of breast cancer incidence and mortality [63]. ~~Breast cancer patients receiving treatment at an integrative cancer clinic, who were found to have extended survival (38 months versus 16-23 months at comparison clinics), had been counseled to regularly consume soy foods, although not concentrated isoflavones, as part of a whole foods diet [40].~~ However, findings from animal studies [64]of negative effects of the soy isoflavone genistein on breast cancer and its treatment suggest that a simplistic reduction of soy to its major phytochemicals is unwarranted – a caution that should be applied to other foods and herbs as well.

At all levels of investigation, the multi-targeted nature of phytochemicals as well as the integrative therapies is notable. Many isolated phytochemicals and herbal ~~extracts contain multiple phytochemicals, and dietary or biobehavioral interventions as well as exercise~~ may alter large numbers of targets through multifaceted effects on physiology and metabolism [65-67]. A basic complication of these multi-targeted agents, however, is the lack of mechanistic understanding and scientific acceptance of the roles of synergistic or additive molecules in formulation. Although used by human populations for millennia, there remains a question of how to develop and assess multi-component natural product formulations that are suitable for large-scale production. Genome-wide screening for assessment of targeted effects and experimentation with formulation of some herbs typical of traditional Ayurvedic medicine have recently been attempted in Asian laboratories, and are an example of attempts to better understand effects of multi-component agents [68-70].

3. Hallmarks of cancer

In this section we provide brief summaries of each hallmark review included in this special issue of *Seminars in Cancer Biology*. Each summary includes the targets and approaches selected in the hallmark review. Targets and approaches, along with cross-validations, are summarized in Tables 1 and 2. A discussion of the cross-validation results follows. In addition, a summary of the impacts of integrative therapies on cancer-related molecular targets follows the hallmark summary material.

The hallmark summaries are roughly sequenced to capture the acquired capabilities of most cancers (see Figure 2). The section begins with *genomic instability*, an enabling characteristic, followed by *sustained proliferative signaling* and *evasion of anti-growth signaling*, two hallmarks that ensure that proliferation is unabated in cancer cells. These are followed by *resistance to apoptosis* and *replicative immortality*, two layers of defense that are believed to be bypassed in all cancers. Then we discuss *deregulated metabolism and tumor-promoting inflammation*, which signal an important self-reinforcing evolution in the tumor microenvironment. Sections on *angiogenesis* and *tissue invasion and metastasis* speak to disease progression. Finally the *tumor microenvironment* and *immune system evasion* summaries relate to the last lines of defense to be defeated in most cancers.

[Figure 2 about here]

3.1 Genomic instability

Genomic instability plays a critical role in cancer initiation and progression. It provides the means by which a cell or subset of cells acquire a selective advantage over neighboring cells, enabling outgrowth and dominance in the tissue micro-environment. In normal cells, the fidelity of the genome is protected at every stage of the cell cycle by checkpoints. In cancer, the presence of aneuploid cells indicates the failure of one or more of these checkpoints. The resulting genomic heterogeneity may offer the cancer “tissue” growth advantages under

selective pressures, including hypoxia, immune- and therapy-related challenges. Understanding these checkpoints, and how they are bypassed in cancer cells, may provide opportunities for the development of rational combinatorial or spectrum treatment strategies, including nutraceuticals such as resveratrol [71,72].

A cell, either transformed or normal, must pass through multiple checkpoints during the process of division. These checkpoints are operated by functional complexes of proteins that either enable the cell to pass through the checkpoint (e.g. proto- or oncogenes) or prevent the progression through the cell cycle (i.e. tumor suppressors). The abundance of these proteins, and their functionality, can be modified by genetic changes to their encoding sequences or by non-genetic, or epigenetic, changes that regulate their abundance. Briefly, small changes to the genes that encode proto-oncogenes or tumor suppressors will positively or negatively impact the function of the gene products. These small changes can be induced by environmental and lifestyle factors, such as toxic substances, diet, and smoking, or they can be encoded in the individual at conception. In the case of DNA damage generated by the environment, it is important that the cell repairs the damage effectively. Dysfunction in the molecules that come together to recognize and respond to sites of damage is often associated with human cancer. Thus, an understanding of the genetic or epigenetic status of DNA repair genes, and of the nutraceuticals that may modulate them [73], provides an opportunity to predict, detect, prevent and treat a variety of human cancers.

Growing evidences show that vitamins, minerals, and other dietary factors have profound and protective effects against cancer cells, whether they are grown in the lab, in animals, or studied in human populations. In our review, we identify and discuss five priority targets against genomic instability: (1) prevention of DNA damage; (2) enhancement of DNA repair; (3) targeting deficient DNA repair; (4) impairing centrosome clustering; and, (5) inhibition of telomerase activity. Moreover, we highlight vitamin D and B, selenium, carotenoids, PARP inhibitors, resveratrol, and isothiocyanates as priority approaches against genomic instability; these approaches may dampen other enabling characteristics of tumor cells, such as replicative immortality, evasion of anti-growth signaling, tumor promoting inflammation, and oncogenic metabolism [71,74-80].

3.2 Sustained proliferative signaling

Proliferation is an important part of cancer development and progression. This is manifested by altered expression and/or activity of cell cycle related proteins [81,82]. Constitutive activation of many signal transduction pathways also stimulates cell growth. Early steps in tumor development are often associated with a fibrogenic response and development of a hypoxic environment [83,84] which favors the appearance, survival and proliferation of cancer stem cells (CSCs). Part of the survival strategy of CSCs may involve alterations in cell metabolism (such as higher antioxidant levels), and a lack of cell differentiation, which distinguish CSCs from normal tissue stem cells [81,82]. These occur prior to the appearance of tumor, as cells adapt to their changing microenvironment in affected tissue. A part of this adaptation embodies epigenetic and genetic alterations in gene expression [4,85] that also confer resistance to many

cytotoxic treatments [86,87]. Thus, adaptive resistance is likely acquired early in the pathogenesis of many tumor types.

Once tumors appear, the continued selection of cells with sustained proliferative signaling further promotes tumor heterogeneity. This is accomplished by growth and metastasis, which may be supported by overproduction of appropriate hormones (in hormonally dependent cancers), by promoting angiogenesis, by undergoing epithelial-to-mesenchymal transition (EMT), by altering the balance between apoptosis, necrosis and autophagy, and by taking cues from surrounding stromal cells. A number of natural compounds (such as EGCG) have been found to inhibit one or more pathways that contribute to proliferation [88-90]. Many of these compounds are nontoxic at doses that inhibit tumor growth and/or prevent the appearance of tumor. However, one of the keys to their efficacy involves their earliest possible therapeutic application. This is because their efficacy is likely to be the greatest in target tissues prior to the appearance of a tumor where cellular heterogeneity is the least. In addition, many of the steps in carcinogenesis prior to tumor appearance are epigenetic in nature, and are more easily targeted by existing compounds, most of which target wild type molecules. This approach limits adaptive resistance, since early intervention does not have to deal with the issues of aneuploidy, loss of heterozygosity in multiple tumor suppressor genes, and point mutations in oncogenes. The contribution of bioinformatics analyses will be important for identifying signaling pathways and molecular targets that may provide early diagnostic markers and/or critical targets for the development of new drugs or combinations that block tumor formation. Thus, early intervention in pathways and molecules that mediate sustained proliferative signaling will limit adaptive resistance because it targets cells in tissues that have limited genotypic and phenotypic heterogeneity.

Targets selected for sustained proliferative signaling are HIF-1 signaling, NF-κB signaling, PI3K/Akt signaling, Wnt (β-catenin) signaling, IGFR1 signaling, cell cycle (CDKs/cyclins), androgen receptor signaling, and estrogen receptor signaling. Possible therapeutic approaches include curcumin, genistein and resveratrol.

3.3 Evasion of Anti-growth Signaling

Normal cells must acquire the ability to continuously proliferate in order to transform into malignant phenotypes. However, cells have internal programs (anti-growth signaling) to oppose limitless growth. In order to continue to proliferate, cancer cells must somehow evade many anti-growth signals. In general, anti-growth signaling is mediated by the activation of tumor suppressor genes. The Cancer Genome Atlas has compiled data encompassing all tumor types, which indicates that *p53* is the most frequently mutated tumor suppressor gene followed by *PTEN, APC, ATM, BRCA2*, *VHL*, *RB*, *CDKN2A, BRCA1* and *WT1*.

RB1 was the first identified tumor suppressor and deletion of this gene is frequently found in cancers [91]. In many cases, the loss of RB is due to defects in upstream signaling molecules such as inactivation of INK4. Loss of p16ink4a results in unopposed activation of CDK4/6, which

phosphorylate the RB protein thereby activating E2F-mediated transcription of genes involved in entry into the cell cycle [92].

Another tumor suppressor frequently deleted due to chromosomal loss is p53 [93]. In fact, more than 50% of all tumors have loss of p53 tumor suppressive functions. Recently, mutant p53 has gained renewed attention due to the fact that along with the loss of tumor suppressive functions, mutant p53 gains oncogenic/tumor promoting functions [94]. Epigenetic silencing of tumor suppressor proteins, which includes DNA methylation, histone methylation and acetylation, is another mechanism through which tumor cells evade anti-growth signaling. Many tumor suppressor genes have been found to have promoter hypermethylation in cancers [95]. Finally, anti-growth signaling plays a major role in treatment response and drug development. For example, the patients with HPV-positive oropharyngeal cancer mostly retain wild-type p53 and have better prognosis and survival. Although genetic alterations are mostly irreversible, epigenetic repressions are potentially reversible and target for drug development. At least three HDAC inhibitors, belinostat, vorinostat and romidepsin, are currently approved by the U.S. FDA for cancer treatment. Many natural compounds also target the restoration of tumor suppressors through modifying epigenetic changes [96-100]. Thus, approaches to activate anti-growth signaling will open another chapter for cancer prevention and therapy.

The prioritized targets for anti-growth signaling are the RB, p53, PTEN, Hippo, GDF15, ARID1A, Notch, IGF-1R and others. The approaches are inactivation of E2F by down regulation of pRb using CDK inhibitors, activation of p53 through up-regulation of wild-type p53, activation of PTEN to inhibit PI3K-AKT, activation of Hippo pathways by inhibiting YAP/TEAD activity, induction of GDF15 through p53 activation, activation of ARID1A, blocking NOTCH pathway, and inhibition of IGF-1R to restore tumor suppressor pathways. Furthermore, while the evasion of anti-growth signaling is a critical hallmark of cancer, other hallmarks are similarly important and a more integrative approach is necessary to simultaneously target several hallmarks of cancer to combat this deadly disease.

3.4 Resistance to apoptosis

Apoptosis is a natural way of removing aged and unhealthy cells from the body [101]. However, in cancer, cells lose their ability to undergo apoptosis leading to uncontrolled proliferation and multiplication. These malignant cells are often found to over express many of the proteins that play important roles in resisting the activation of the apoptotic cascade and one of the major hallmarks of human cancers is the intrinsic or acquired resistance to apoptosis [102]. Evasion of apoptosis may contribute to tumor development, progression, and also to treatment resistance, since most of the currently available anticancer therapies including chemotherapy, radio- and immunotherapy primarily act by activating death/apoptotic pathways in cancer cells [103]. Hence, a better understanding of the molecular mechanisms underlying tumor resistance to apoptotic cell death is expected to provide the basis for a rational approach to develop molecular targeted therapies.

Apoptosis resistance is multi-factorial and emanates from the interactions of various molecules and signaling pathways at multiple levels. Several mechanisms exist allowing cells to escape programmed cell death. Among them is the over expression of the anti-apoptotic molecules. The review begins with discussing how B-cell lymphoma-2 (Bcl-2) family proteins play a critical role in the biology of apoptosis resistance. Comprehensive information is presented in regards to the success and challenges in the development of robust agents against the Bcl-2 homology domain 3 (BH3) proteins and how these agents have accelerated toward clinical application. Other cell death mechanisms such as autophagy and necrosis are also discussed and the strategies; in particular, the use of natural agents such EGCG is highlighted. The role of the chaperone protein heat shock protein 70 (Hsp70) in apoptosis resistance is evaluated and suggestions to overcome this critical protein marker using natural products are presented. The article also discusses the molecular mechanisms that support the resistance to apoptosis in different disease models such as glioblastoma, multiple myeloma and chronic lymphocytic leukemia. The role of epigenetic players, particularly the non-coding RNAs/ microRNAs (miRNAs), is also elaborated. The article also touches upon novel targets such as ecto-nicotinamide dinucleotide disulfide thiol exchanger protein (ENOX) and nuclear export protein chromosomal regional maintenance protein 1(CRM1), along with specific strategies to overcome these important drug resistance promoters. Other targets selected include inhibition of Mcl-1, activation of tumor autophagy, activation of tumor necrosis, inhibition of Hsp90, inhibition of proteasomes, and inhibition of EGFR and Akt. Approaches to these targets include gossypol, UMI-77, EGCG, triptolide, PXD, selinexor, and inhibitors of EGFR and Akt. Collectively, the knowledge gained through greater understanding of the apoptosis resistance targets and specific strategies is anticipated to bring forward a broad form of therapy that could result in better treatment outcome in patients suffering from therapy-resistant cancers.

3.5 Replicative immortality

Replicative immortality, the ability to undergo continuous self-renewal, is necessary for propagation of normal germ cells, but is not a property of normal somatic cells. When acquired by somatic cells that have sustained genetic damage or instability, replicative immortality allows accumulation of sequential aberrations that confer autonomous growth, invasiveness, and therapeutic resistance [104]. As a result, several mechanisms have evolved to regulate replicative potential as a hedge against malignant progression [105]. Senescence, a viable growth arrest characterized by the inability of affected cells to resume proliferation in the presence of appropriate mitogenic factors, is a specific response to the gradual shortening of chromosomal end structures (telomeres) with each round of cell replication, and a more general response to oncogenic and genotoxic stresses. Senescence often involves convergent interdependent activation of tumor suppressors p53 and p16/pRB [106,107], but can still be induced, albeit with reduced sensitivity, when these suppressors are inactivated. Doses of conventional genotoxic drugs required to achieve cancer cell senescence are often much lower than doses required to achieve outright cell death [108]. Additional targeted therapies may induce senescence specifically in cancer cells by blocking cyclin-dependent kinase mediated inhibition of RB-family proteins [109], or by exploiting cancer cells' heightened requirements for maintenance of telomere length through the action of the enzyme telomerase [110].

Developing optimized and truly holistic cancer prevention and treatment regimens will likely incorporate strategies that target replicative immortality.

The chief advantage to be gained by the use of senescence-inducing therapeutic regimens is elimination of the tumor's repopulating ability with reduced collateral damage compared to conventional cytotoxic regimens. There are, however, certain questions and risks associated with this strategy that must be addressed before its clinical adoption. In the case of telomere and telomerase based strategies, replicative senescence may occur more readily in rapidly dividing cancer cells bearing short telomeres than in slowly dividing stem cells with comparatively longer telomeres, but telomere lengths in cancer cells may still be long enough to permit sufficient population doublings for invasion and metastases to occur [110]. Moreover, telomere dysfunction promotes the development of chromosomal instability, which in turn can generate mutations that enable cells to become drug resistant and/or activate alternative lengthening of telomeres (ALT) mechanisms for telomere maintenance and/or become more malignant [111]. High priority should therefore be given to further research into the determinants of senescence stability, as the implications of delayed cell cycle re-entry, permanent cytostasis, or eventual clearance may be profoundly different. Lower doses of genotoxic drugs needed to induce senescence may reduce collateral damage to critical normal cells, but allow establishment of dormancy and/or adaptive resistance by cancer cells. The microenvironmental and systemic effects of senescent cells also need further clarification, as factors secreted by senescent cells may promote tumorigenic changes in nearby cells. Conversely, since it is almost impossible to kill all the cells in malignant tumors even using the highest tolerated doses of chemotherapy, combined use of an agent that induces or enhances stable senescence in the cancer cells that manage to retain viability might additively or synergistically increase therapeutic efficacy.

A number of potential targets can be singled out for further research, including telomerase, hTERT, mTOR, CDK4/6, CDK 1/2/5/9, Akt and PI3K. Several approaches deserve further research; in particular, the activity of the phytochemicals is still far from clinical utility. These include imetelstat, genistein, perillyl alcohol, palbociclib, dinaciclib, curcumin and EGCG.

3.6 Deregulated metabolism

Deregulated metabolism is a hallmark of cancer, where many cancer cells show increased glucose uptake and produce lactate. This observation is often called the "Warburg effect" [112], but how and why cancer cells reprogram their metabolic state is not well understood. Recent research has focused on understanding the metabolic changes accompanying oncogenesis [24]. A new model of cancer metabolism positions metabolic rewiring in cancer as a coordinated process to support rapid cellular proliferation by tuning cellular energy production needs towards biosynthetic processes. Indeed, several metabolic shifts associated with cancer can be linked to cellular growth, which serve to support biosynthesis of lipids, proteins, nucleic acids required for tumor formation and survival [113].

In several cases, expression of oncogenes and/or loss of tumor suppressors lead directly to changes in metabolism, by expression, activity, or flux of key metabolic nodes. Several components of glucose and glutamine metabolism have emerged as important regulators of metabolism in cancer. In glucose metabolism, hexokinase 2 (HK2), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), pyruvate kinase isoform M2 (PKM2) all regulate glycolytic flux. Using a "kitchen sink" analogy for glycolysis, both HK2 and PFKFB3 are regulators of the faucet, and fill up the sink. Conversely, PKM2 regulates the drain. Cancer metabolism turns on the faucet and plugs the drain, which over-spills the glycolytic pathway and provides metabolites used as building blocks for cellular growth. Efforts are underway to identify therapeutic strategies to "turn off the faucet" or "unplug the drain" in glycolysis, limiting cellular growth in cancer. Recent studies have also determined that glutamine is used as a fuel (glutaminolysis) in proliferating cancer cells. Glutamine oxidation can provide carbon and nitrogen for growth, and therefore is an attractive therapeutic target in cancer. Additionally, mutations in genes encoding enzymes directly involved in metabolic pathways have been associated with several types of cancer. Rather than acting as a bystander or facilitator of oncogenesis, aberrant metabolism now has a pro-oncogenic role and has led to the redefinition of some metabolites as 'oncometabolites' [114]. Indeed, these oncometabolites are powerful influencers of proliferation, and are also positioned as new therapeutic targets.

In principle, a broad-spectrum approach to target metabolic shifts in cancer is likely to be a promising therapeutic strategy. However, studies using this approach to target deregulated metabolism in cancer are in their infancy. Lessons could be learned from other strategies to target mitochondria or to target metabolism in order to identify efficacious and safe therapies targeted at cancer metabolism; some drugs targeting metabolism are being re-purposed for their anti-tumorigenic effects. Several approaches have been suggested, including 3-bromopyruvate, PFK-15, TEPP-46, dichloroacetate, hexachlorophene, BPTES and FX11, but data for these must be regarded as extremely preliminary, and they lack sufficient justification to be included in therapy without further study. Most target proteins or pathways identified as having potential to manipulate cancer metabolism have not been directly tested in the context of other hallmarks. The emerging efficacy of physiological interventions that manipulate cancer outcomes, such as fasting, calorie restriction, or exercise, could influence cancer metabolism and other hallmarks of cancer [115]. Future studies directly testing the ability to manipulate deregulated metabolism in cancer will be an important and exciting new area of cancer biology and has potential for treating a variety of cancers.

3.7 Tumor promoting inflammation

Virchow first proposed the role of inflammation in cancer in 1863, while observing the presence of leukocytes in neoplastic , and empirical evidence has since underscored the importance of this linkage [116,117]. The inflammatory milieu promotes a cellular microenvironment that favors the expansion of genomic aberrations and the initiation of carcinogenesis [118]. Chronic inflammation is linked to various phases of tumorigenesis, such as cellular proliferation, transformation, apoptosis evasion, survival, invasion, angiogenesis and metastasis [119-121].

Inflammation is also known to contribute to carcinogenesis through the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which can damage DNA at the site of the tumor [122]. Free radicals and aldehydes, produced during chronic inflammation, can also induce deleterious gene mutation and post-translational modifications of key cancer-related proteins [123].

In addition, chronic inflammation has an influence on immune system constituents that are directly linked with cancer progression. Under normal conditions, immune cells, including macrophages, granulocytes, mast cells, dendritic cells, innate lymphocytes, and natural killer (NK) cells serve as the front line of defense against pathogens. When tissue disruption occurs, macrophages and mast cells secrete matrix-remodeling proteins, cytokines and chemokines, which activate local stromal cells (fibroblasts, adipocytes, vascular cells, etc.) to recruit circulating leukocytes into damaged tissue (acute inflammation), to eliminate pathogens [124]. However, when these processes are initiated in the tumor microenvironment, they are not resolved, which leads to chronic inflammation of the "damaged" (tumor) tissue. Thus, while acute inflammation normally supports and balances two opposing needs for the repair of damaged tissues (apoptosis and wound healing), chronic inflammation represents a loss of this balance and the resulting confluence of factors has deleterious implications for the immune system [125].

Accordingly, the relationship between tumor-promoting inflammation and cancer is important to consider. So we identified macrophage migration inhibitory factor, cyclooxygenase-2, NF-κB, tumor necrosis factor alpha (TNF-α), inducible nitric oxide synthase, protein kinase B, and chemokines as important anti-inflammatory targets that might be suitable for a multi-pronged therapeutic approach to inflammation suppression. Additionally, we focused on curcumin, resveratrol, EGCG, genistein, lycopene, and anthocyanins, as forms of low-cost chemistry with little to no toxicity that could be employed to reach these targets.

Future translational work should make use of promising agents such as these (combined as constituents within a multi-pronged anti-inflammatory approach) bearing in mind that some of these targets impact the immune system and can increase the risks associated with infection. Bioavailability challenges are also a concern for a number of these agents but recent advances in delivery systems will help address this issue.

3.8 Angiogenesis

Angiogenesis, the expansion of an existing vasculature, is the main mechanism of blood vessel growth in adults, and is therefore essential for tumor development [126].Tumor angiogenesis is switched on by changing the balance between angiogenic factors and inhibitors in favor of angiogenesis [127], a process induced by tumor hypoxia as the tumor grows beyond a size of approximately 1 mm3 [126,128]. At more advanced stages, progressive genomic instability in the tumor leads to mutations in pathways regulating the production of multiple angiogenic factors [129], and stroma cells, also become important sources of sustained angiogenic factor production [130]. These collectively result in a stronger and more complex angiogenic factor

profile. It is therefore not surprising that targeted neutralization of a single angiogenic factor, which has been the focus for anti-angiogenic cancer therapy so far, rarely produce long-term, anti-tumor effects [130].

Due to the multifactorial nature of tumor angiogenesis this process is likely to be more efficiently treated by targeting multiple aspects of tumor angiogenesis and vascular dysfunction at the same time. In our review on broad targeting of angiogenesis for cancer prevention and therapy in this issue of Seminars in Cancer Biology, we have identified and discussed 10 of the most important targets for tumor angiogenesis and vascular dysfunction, namely to inhibit endothelial cell migration/tip cell formation, reduce structural abnormalities oft tumor vessels, reduce hypoxia, inhibit lymphangiogenesis, reduce elevated interstitial fluid pressure, reverse poor perfusion normalize disrupted circadian rhythms, suppress tumor promoting inflammation, deactivate tumor promoting fibroblasts and normalize tumor cell metabolism/acidosis.

Currently available non-specific anti-angiogenic agents, able to perform some of these tasks, are however quite toxic, which render them unsuitable for long-term use [129,131,132]. There is an urgent need to identify alternative compounds that could be used in combination over extended periods of time, targeting tumor angiogenesis broadly and thus lowering the risk of resistance. Plant-derived compounds, phytochemicals, are in many cases better tolerated than the synthetic analogues used in cancer therapy today. Furthermore, they often exhibit broader mechanisms of action and sometimes even higher affinity against important cancer targets compared to the synthetic alternatives [133]. In our review we discuss evidence supporting phytochemicals as anti-angiogenic agents and suggest how these could be combined for maximum effect with minimum toxicity in treatment of cancer. In particular we identify 10 phytochemicals which would be effective as approaches to neutralize the 10 identified targets: oleic acid, tripterine, silibinin, curcumin, EGCG, kaempferol, melatonin, enterolactone, withaferin A and resveratrol. Finally we discuss the optimal use and combination of these phytochemicals in anti-angiogenic therapy focusing on delivery, toxicity and their use in prophylactic regimens.

3.9 Tissue invasion and metastasis

Cancer is a key health issue across the world, causing substantial patient morbidity and mortality. Patient prognosis is tightly linked with metastatic dissemination of the disease to distant sites, with metastatic diseases accounting for a vast percentage of cancer patient mortality [24,134,135]. In order to successfully disseminate to and establish at a secondary location cancer cells must overcome several obstacles as they progress through the metastatic cascade. Successful progression through this cascade is linked with numerous established changes in cellular functions leading to the acquisition of an invasive phenotype. This involves loss of cell-cell contact with the main tumor body, invasion, degradation and migration through surrounding tissue and extra cellular matrix (ECM), secretion of angiogenic / lymphangiogenic factors and intravasation to the blood / lymph vessel, transport around the body and evasion of

the immune system, extravasation at the secondary site and establishment of a secondary tumor [136,137].
Hence, factors influencing these processes such as cell adhesion molecules (CAMs), proteolytic matrix degrading enzymes, cell motility and factors involved in the process of EMT have all been subject to scientific scrutiny. Additionally, the complex heterogeneity within tumors, together with cellular interactions between tumor cells and other, non-cancerous, cell types have been established to play key roles in metastatic dissemination and add further complexity to this cascade [135,137]. While advances in the field of cancer research have been made, the process of cancer metastasis and the factors governing cancer spread and establishment at secondary locations are still poorly understood. Current treatment regimes for metastatic disease pose many adverse effects, which can further negatively impact on a subset of patients generally presenting with poorer health conditions. Hence there is a great need to develop new therapeutics that not only target tumor growth and inhibit metastasis but that also have a lower toxicity and reduced inherent side effects. Factors associated with metastasis such disruption of E-cadherin and tight junctions, key signaling pathways, including uPA, PI3K/AKT, FAK, β-catenin/ZEB-1 and TGF-β, together with inactivation of AP-1 and suppression of MMP-9 activity should be considered as key research priorities.
Here, the need is highlighted for new, low toxicity compounds, which interfere with these processes but remain inexpensive alternatives that are readily available and free from intellectual property. Phytochemicals, or natural products, such as those from *Agaricus blazei, Albatrellus confluens, Cordyceps militaris, Ganoderma lucidum, Poria cocos* and *Silybum marianum*, together with diet derived fatty acids gamma linolenic acid (GLA) and eicosapentanoic acid (EPA) and inhibitory compounds have potential to inhibit these key metastatic events. These potential targets and strategies thus present new therapeutic opportunities to both manage cancer metastasis as well as having holistic effect against many of the hallmarks of cancer.

3.10 Tissue interactions in the tumor microenvironment

Cancer arises in the context of an in vivo tumor microenvironment. This microenvironment is a cause and consequence of tumorigenesis that consists of cancer cells and host cells that co-evolve dynamically through indirect and direct cellular interactions, produced metabolites and secreted factors [138,139]. In turn, this environment regulates the ability of a cancer to grow and survive via multiscale effects on many biological programs including cellular proliferation, growth and metabolism, as well as angiogenesis and hypoxia, innate and adaptive immunity [140]. We have identified specific biological programs that could be, based on our most recent understanding, exploited as targets for the prevention and therapy of cancer, including: the inhibition of cholesterol synthesis and metabolites, reactive oxygen species and hypoxia, macrophage activation and conversion, regulation of dendritic cells, regulation of angiogenesis, fibrosis inhibition, endoglin, and cytokine signaling. These programs emerge as examples of important potential nexuses in the regulation of the tumorigenesis and the tumor microenvironment that can be targeted.

The targets we identified include metabolic programs that may broadly influence many cell biology programs that impact tumorigenesis and the tumor microenvironment (cholesterol synthesis and metabolites, reactive oxygen species (ROS) and hypoxia), inflammation, innate and adaptive immunity related programs (macrophage conversion, dendritic cell activation, immune signaling), host microenvironment associated cellular programs (fibrosis, angiogenesis), and cytokine mediated regulatory programs (IL-6, endoglin, and JAK). We particularly focused on identifying approaches for inhibiting these targets that included natural products that have been suggested to have significant anticancer activity. Some of these molecules may more generally influence tumorigenesis and the microenvironment (berberine), others more specifically target reactive oxygen species (ROS; resveratrol, desoxyrhapontigenin) macrophage conversion (onionin A), indoleamine 2,3-dioxygenase (IDO) regulation of dendritic cells (EGCG), cholesterol synthesis (genistein), fibrosis (naringenin), inflammation and immune signaling (piperine) and JAK signaling (zerumbone). We believe that our approach will provide a starting point for examining synergies that might be anticipated in testing certain targets and/or mixtures of natural chemical constituents that may modulate the tumor microenvironment in the treatment and prevention of cancer.

3.11 Immune system evasion

Tumors evade immune attack by several mechanisms including generation of regulatory cells and their secretions, defective antigen presentation, induction of immune suppressive mediators either by cancerous cells themselves or by those in the microenvironment, tolerance, immune deviation and apoptosis.

Current approaches to immune therapy include a) cellular targets, b) molecular targets, c) vaccination therapy, d) therapy by phytochemicals, e) adoptive T cell therapy and f) immunomodulatory antibodies. Of these anti-cancer agents, the most important are those that are targeted in nature and to lesser extent, those that are non-specific in nature. Targeting specific costimulatory molecules such as cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) [141]or programmed cell death protein (PD1/PD-L1) [142]is considered an important anticancer strategy. Of the immunomodulatory antibodies, only anti-CTLA-4 (ipilimumab) has been approved for clinical use in the USA, Canada, United Kingdom, and European Union for melanoma. Also, anti-PD-1 antibodies are showing enormous therapeutic potential in advanced cancers. Targets that are considered appropriate for broad-spectrum, low-toxicity therapeutics are less specific and include enhancing Th1 responses, enhancing γδ T-cells, activation of macrophages, inhibition of Treg lymphocytes, enhancing natural killer cell activity and induction of IL-12.

There are a number of important non-specific anti-cancer agents that have been reported including vaccination therapy, as well as non-specific bacteria-based therapies [143], and phytochemicals [144-146]. Phytochemicals (the biologically active components of fruits and vegetables) have been shown to exert protective effects against cancer. Examples of potential phytochemical approaches include extracts of *Ganoderma lucidum*, *Trametes versicolor*,

Astragalus membranaceus, and *Lentinus edodes*, as well as astaxanthin and the polyphenol resveratrol analogue HS-1793. There is, however, a downside to phytochemical therapy such as their poor absorption by humans and rapid metabolism and excretion. More work is required to assess which phytochemicals block evasion of immune surveillance and also to determine which phytochemicals promote antitumor responses in cancer patients before these can be recognized for therapeutic value in the clinic.

3.12 Summary of findings on targets and approaches in hallmark reviews

~~What are the targets and approaches that were found in each hallmark review, and how do they interact with each other? Has the review process resulted in a group of targets and approaches that work at cross-purposes, so that choosing one group of targets for a broad-spectrum approach will entail altering the activity of other targets, resulting either in no activity or, worse, a negative effect on patients' well-being, by stimulating proliferation of cancer cells? The cross-validation process used in the preparation of this set of reviews is an attempted to investigate the interactions of prioritized targets and approaches with the array of hallmarks. More detailed discussion of these interactions can be found in the individual hallmark reviews. As described above, each interaction was assessed as complementary (enhances cancer prevention or treatment), contrary (counteracts cancer prevention or treatment), no known relationship or controversial (both complementary and contrary interactions noted in the literature).~~ As described above, a cross-validation process was employed to review the proposed actions on each target and all of the approaches for known effects on other hallmark areas and the tumor microenvironment. Anti-carcinogenic synergies and confounding/pro-carcinogenic effects were then compiled and summarized in Tables 1-3. ~~Tables 1-3 summarize the cross-validations in the hallmark reviews~~. Supplemental tables S1 and S2 contain the aggregated cross-validation tables from each review (with references omitted). More detailed discussion of these interactions can be found in the individual hallmark reviews.

[Tables 1, 2 and 3 about here]

Table 1 shows an alphabetical listing of prioritized targets from each hallmark review, as well as the number of ~~complementary,~~ contrary, controversial none known and complementary ~~controversial~~ interactions with all other hallmarks. Note that deregulated metabolism targets do not appear in the table; too little is known about the targets in this new area of research to reliably assess their interactions with other hallmarks. Of these relationships, only 3.5% were contrary, 7.8% were controversial, 21.9% of interaction assessments found no known relationship, and 66.7% were complementary.

Table 2 shows the prioritized therapeutic approaches – the phytochemicals, plant extracts and drugs chosen as modifiers of the priority targets. Of these, 0.9% were contrary, 5.7% were controversial, 31.8% had no known relationships and 61.7% were complementary. Both contrary and controversial interactions indicate potential conflict among the targets and approaches selected for different hallmarks that could result in a broad-spectrum approach with antagonistic, rather than synergistic effects.

The small number of contrary and controversial interactions is encouraging, and suggests that the potential for negative interactions among the selected targets and approach may be limited. However, this may also reflect the common bias in the literature to publish positive antitumor effects. Nearly a third of potential interactions were listed as having no known relationship, suggesting the need for substantially more research in this area. The large number of complementary interactions is also encouraging but may result from indirect or bystander effects as discussed below.

Table 3, in which the different types of interactions of both targets and approaches are listed for each hallmark, also shows some interesting trends. Genetic instability has the largest number of apparent null relationships with the targets and approaches. On the other hand, tumor microenvironment, tissue invasion and metastasis and resistance to apoptosis have the highest number of complementary interactions for both targets and approaches, whereas tumor-promoting inflammation and angiogenesis have the highest number of contrary interactions.

There are a number of limitations that should be noted in this delineation of cross-hallmark relationships. First, the researchers who assembled these results were not asked to distinguish between direct effects on other hallmark areas and reported effects on other hallmark areas that may have resulted in an indirect or "bystander" effect mediated through a different mechanism. In many cases, but not all, this distinction was made. Therefore it is likely that some of the complementary interactions do not represent a fully independent cross-hallmark relationship, but rather are simply indicative of some sort of downstream effect (e.g., within a signaling cascade or via some other signaling molecule that exerts pleiotropic effects). However, we did not feel that this project needed to investigate the nature of these complementary interactions in detail. ~~although researchers may wish~~ ~~We urge researchers to undertake such an assessment on their own prior to translational work.~~ Instead, our main concern was focused on the possibility that a large number of cross-hallmark relationships might be revealed where actions with pro-carcinogenic or tumor-promoting potential had been reported. ~~The identification of~~ It was more important to identify contrary and controversial cross-hallmark interactions than complementary ones, ~~was therefore of greater importance, since targets or approaches that exert pro-carcinogenic actions should likely be avoided in developing agents or interventions for testing.~~ since targets or approaches that exert pro-carcinogenic actions would normally need to be more carefully assessed (or avoided altogether) in the development of combination approaches or interventions.

The second limitation of these reports of cross-hallmark relationships is related to data quality. In some instances, the underlying evidence used to support the indication of a cross-hallmark relationships was robust, consisting of multiple studies involving detailed *in vitro* and *in vivo* findings. However, in other instances, the underlying evidence that was used to report the existence of a cross-hallmark relationship was quite weak (e.g., consisting of only a single i*n vitro* study involving a single cell-type). Again, the overarching goal in this project was to create a foundation that would allow us to look systematically across the literature in each of these

areas, to help us shape the selection of the targets and approaches. So although we realized that not all of these reports of cross-hallmark relationships represented the same level of evidence, we still wanted to examine available evidence to flag targets and approaches where pro-carcinogenic actions had been reported.

There was also considerable debate within the task force over the value of tables containing only a simplified indication of a relationship (i.e., + or -) supported by evidence that varied considerably in quality. But since many individual studies and reviews that focus on therapeutic approaches fail to work systematically across the spectrum of incidental actions that might result from combining therapies, it was our opinion that a tabularized framework was the only way to ensure that we had assembled a complete view of ~~incidents of~~ cross-hallmark activity.

The types of approaches selected differed among different review teams. While some review teams selected all or mostly phytochemicals or plant extracts, some teams felt that the evidence for these was insufficient, and emphasized other types of molecules, including drugs in development. These may pose more difficulties for translational investigators due to intellectual property, toxicity or other concerns, but may offer advantages in a more clear understanding of their mechanisms. We suggest, however, that the approaches as well as the targets presented in Tables 1 and 2 can be viewed as simply a model for broad-spectrum cancer therapies, rather than as a conclusive or final list. Some of the recommended approaches are clearly experimental, and further research will likely discover compounds, phytochemical or synthetic, that are not on this list that may be useful in a broad-spectrum approach.

Bioavailability of the phytochemicals chosen will also be a concern for future studies. However, the need for development of better preclinical models for screening compounds and testing rationally designed combinatorial therapies composed of compounds from any source is obvious, and should clearly be the first step in the development of the broad-spectrum approach.

3.13 Role of integrative therapies in the broad-spectrum approach

Integrative medicine is an approach to health and healing that “makes use of all appropriate therapeutic approaches, healthcare professionals and disciplines to achieve optimal health and healing” [147]. A comprehensive integrative medicine intervention for cancer patients typically includes nutrition education, mind-body medicine and physical activity components, as well as dietary supplements including herbs, nutraceuticals and phytochemicals [34,148]. Such an intervention may contribute uniquely to a broad-spectrum therapeutic approach through its impact on a wide variety of relevant molecular targets and hallmarks. Hallmarks that may be particularly impacted include genomic instability, tumor-promoting inflammation, deregulated metabolism and immune system evasion. Because of their susceptibility to manipulation by diet, exercise and supplementation, these may be characterized as metabolic hallmarks.

Nutrition has long been the primary focus of research on integrative interventions for cancer. The World Cancer Research Fund and the American Institute for Cancer Research find that diets

high in fruits and vegetables substantially reduce risks of several cancers [149]. ~~Several lines of research have suggested that diets similar to those espoused for~~ Cancer prevention diets are also suitable after a cancer diagnosis [150]. For example, colon cancer patients eating a Western diet after diagnosis were at higher risk for recurrence and mortality than those with healthy diets [151]. ~~Ovarian cancer patients consuming diets high in fruits and vegetables had relatively improved survival, while those consuming diets high in red or processed meats and milk had reduced survival~~. Breast cancer patients who followed low-fat diets were found to have lost weight and had lower recurrence risks, especially among patients with estrogen receptor-negative cancers [152]. Trials of diets enriched in whole grains, low-glycemic diets, and both low-fat diets and Mediterranean diets enriched in olive oil and almonds reduced levels of inflammation as measured by CRP [153-156]. Low fat diets, weight loss and supplements (anthocyanins and fish oil) have been observed in randomized trials to reduce cytokines and signaling molecules ~~such as NF-κB, IL-6 and TNF-α~~ [157-160]. Mind-body interventions have emphasized immune targets, with findings of interventional trials including activation of T-cells and lymphokine-activated killer cells and increased natural killer cell activity [161,162]. Exercise interventions have documented effects on survival, insulin-like growth factor-1, natural killer cell activity, and sex hormones [163-166]. While much work remains to be done on integrative interventions, especially in aiding patients ~~to overcome barriers~~ to adopt lifestyle interventions, these ~~intriguing~~ preliminary data suggest that integrative medicine may ~~have~~ significantly ~~potential to~~ support a broad-spectrum approach to cancer therapy.

4. Proposed research model

The review process for this project has revealed ~~a multiplicity of~~ many potential targets and approaches. The cross-validation activity suggests that ~~the effects of the majority of targets and approaches act on other hallmark areas in a manner that is complementary rather than contrary manner~~ only a small number of targets and approaches affect other hallmarks in contrary or controversial ways. ~~The next question facing participants in this project was how these data might be used as the basis of a research and development program for a broad-spectrum approach (i.e., one that is aimed simultaneously at many high-priority targets).~~ Indeed the results are quite promising and suggest that the design of a broad-spectrum approach should be feasible from a safety standpoint. Although considerable research will be needed, disease relapse is a substantial and longstanding problem, so this novel model definitely warrants further investigation

~~One element of the broad-spectrum strategy, which could be applied in many settings, is the use of multi-component therapeutics based on selections from the natural product and other approaches listed in Table 2. These could be described as multi-targeted agents. The approaches in Table 2 are mostly phytochemicals, but some, such as *Astragalus membranaceus*, *Ganoderma lucidum* and *Lentinus edodes*, are relatively unpurified herbal or fungal extracts.~~

~~In the case of non-characterized (unpurified) natural products, there may be two situations. First an active molecule may have been well-characterized, but is not highly efficacious, or is perhaps toxic when given as a single agent. Second, there may be no single active molecule,~~

but several molecules may work in tandem, synergistically or additively. Current standards in conventional medicine tend to frown upon either of the situations pertaining to unpurified natural products. Even when an unpurified product has been shown clinically to have an effect on a particular disease or symptom that is not placebo it tends to be less well-accepted by the medical community than compounds that are given in isolation. Anticancer effects have, nevertheless, been demonstrated for multi-component, multi-targeted agents. An agent composed of mushroom extracts (*Coriolus versicolor*, *Ganoderma lucidum*, *Phellinus linteus*), herb extracts (*Astragalus membranaceus*, *Scutellaria baicalensis* and turmeric) and phytochemicals (diindolylmethane and quercetin), for example, was shown to inhibit proliferation and metastatic behavior of MDA-MB-231 breast cancer cells *in vitro*, and to reduce volume of implanted tumors as well as markedly reduce metastases *in vivo* [227] .

[Next 4 paragraphs moved to section 4.4, Translational considerations] Assuming that translational research work will involve a substantial combination of therapeutic agents such as those proposed in Table 2 as a starting point, a first step would be the selection of specific targets and approaches for preliminary study. To achieve a truly broad-spectrum effect, one strategy might be to use small doses of every approach that lacks significant contrary interferences. While such a mixture might be made up and applied to cell lines, it could be questioned whether the concentrations that could be achieved in the cells would be physiologically relevant, especially given the low bioavailability of many phytochemicals. In fact, most *in vitro* work on phytochemicals is conducted at concentrations that are not achievable in humans, and the pharmacokinetics and pharmacodynamics of phytochemicals are complex and many are not yet well known, although progress is being made on some agents [169]. Another method to narrow the number of phytochemicals that need to be in an agent might be to select the phytochemicals that are most widely represented across hallmarks, such as curcumin and resveratrol, and analyze combinations of these agents. Some of the selected approaches, e.g. silibinin, appear to have favorable pharmacokinetics [170]. Other phytochemicals with favorable pharmacokinetics could also be considered for inclusion in a broad-spectrum agent, such as phenethyl isothiocyanate [171].

Alternative approaches to the question of bioavailability are being explored, especially with the polyphenols. One of the main issues with these compounds, which include quercetin, green tea catechins, curcumin and others, is ensuring that circulating doses of aglycones (one of the active forms of these molecules), are sufficient for activity. After oral supplementation of food-grade molecules at doses safe for humans (200-500 mg/day), only conjugated forms are found in the bloodstream. As an example, quercetin is not found in the plasma as aglycone or as the parent glycosides: at the doses usually employed in intervention studies, it would be found exclusively as methyl, sulfate or glucuronic acid conjugates [172]. This observation discloses a paradox common to many biologically active phytochemicals: if free aglycones are absent *in vivo* after a dietary intake or supplementation with high doses, how can we explain the high biological activity of these molecules, largely described *in vitro*?

Two main hypotheses can be considered. First, conjugated forms of some flavonoids (e.g. quercetin) may be biologically active. Second, after cellular uptake, these metabolites may be

~~de-conjugated, regenerating the free aglycones. To sustain these hypotheses, key issues need to be addressed, such as the efficacy of mechanisms of uptake of polyphenol metabolites and the substrate specificity of each metabolite, which is largely unknown. The use of pure compounds tested *in vitro* may shed light on these questions. Alternatively, pharmacological doses (2-4 g/day) administered orally [173]may saturate the metabolic pathways of conjugation [172,174]. Efforts are being made, however, to improve bioavailability of these agents, such as microspheres [175], liposomes [176] and nanoparticles [177]. An additional complication is that individuals may vary in their absorption, distribution, metabolism and elimination of phytochemicals, based in some instances on genetic variability [178], dietary habits [179] and potentially on intestinal microbiota [180].~~

~~Considerations of quality control of the final product are essential along the spectrum of research from *in vitro* studies to clinical trials. Good agricultural practice, correct botanical identification and good manufacturing practice are mandatory to prevent adulteration, contamination and toxicity [181]. The example of PC-SPES, a botanical cancer remedy that was found to contain indomethacin, warfarin and synthetic estrogens, leading to its withdrawal from the market in 2002 resulted in greater awareness of the need for a strict approach to quality control [182]. The question of whether mixtures of the selected approaches will have adequate stability, or will be subject to degradation due to interactions among various phytochemicals and extracts is likewise of importance. Separating constituents into different dosage forms may be necessary to avoid such interactions.~~

4.1 *In vitro* research

An array of *in vitro* models is available for preliminary study of broad-spectrum formulas. One question is the suitability of receptor-based assays versus cell-based assays. While receptor-based assays may seem more suitable for targeted therapy research, examining the impacts of a putative agent on a molecule such as NF-κB, which is at the intersection of multiple signaling pathways related to inflammation, might be advised. Cultivated cell lines are valuable for preliminary screening of mixtures, but are, in most respects, ~~more similar to each other than to clinical samples, and thus~~ limited in their predictive ability. Isolated cell lines from clinical samples are an alternative, and use of transformed cancer cells versus non-transformed lines should be discussed. Tissue and organ explants are another useful *in vitro* model.

Basic research on the properties of the natural product and other approaches selected in the reviews needs to continue. ~~Effects of these approaches on gene expression, signaling pathways, epigenetics and, specifically, on the targets selected for each of the hallmarks is needed. Beyond this,~~ The pharmacology of mixtures and combinations of phytochemicals, bioavailability, dose optimization and synergy are among the areas in which research is needed for many phytochemicals [167,168]. ~~Models for study of synergy are well known in pharmacology, and have been applied to natural products~~. However, multicomponent herbal therapies used in traditional and alternative medicine have not received detailed analysis. Network pharmacology could be a means of exploring these presumed synergisms, and efforts are being made to apply this approach to the complex herbal mixtures used in traditional

Chinese medicine [169]. Studies on the pharmacokinetics of herbal extracts and phytochemicals, which often begin at the *in vitro* level, are also needed [170].

In sum, given the complexity that is immediately suggested when combinations of approaches are possible, we strongly recommend that well-coordinated, multi-faceted programs be pursued initially to ensure that the constituent approaches that are selected are well-characterized using *in vitro* models, and that delivery methods that are selected for *in vivo* work receive careful evaluation before animal research is undertaken.

4.2 *In vivo* research

Multiple *in vivo* models for further study of broad-spectrum approaches are also available. Two obvious choices are animal tumor models and human tumor xenografts implanted in athymic mice. While human tumor xenografts have the advantage in predicting effects of agents on human cancer cells, animal tumors offer some interesting choices for chemoprevention studies, since several are induced by exposure to various chemicals. The rodent tumors are questionable, however, in their ability to predict human responses to antitumor therapy. Differences in immunity are one consideration, most obviously with athymic mice but also with other animals. Many other differences are known. ~~Experiments with pathogen-free mice are often criticized for not being relevant to humans. Immunity is not the only difference between mouse models and humans:~~ Rodents and humans, for instance, differ significantly in their blood levels of soy isoflavones after these are administered through a variety of dietary and experimental routes [171]. Isoflavone levels in rodents were 20 to 150 times those in humans, raising questions about the suitability of animals for prediction of phytochemical effects in humans.

Additionally, as shown in different preclinical mouse models, immune ~~as well as~~ and inflammatory responses to cancer ~~are not the same~~ differ in young and old individuals, and many cancer treatments are likely to be less effective at older ages. Combination treatment including immunotherapeutic approaches may be most suitable for older animals. Therefore, there is a strong argument for testing and optimizing combination treatments in suitable model systems before attempting to apply them to cancer patients. The NCI Mouse Models of Human Cancer Consortium [172]has tried to provide the scientific community with accurate, reproducible models of human cancers that can be used in translational and pre-clinical studies. Such improved models could be of great importance for developing combination treatment strategies ~~that are effective not only in younger patients but also in the ever-increasing population of older patients~~. Companion animals, such as dogs and cats, ~~can also serve as~~ which experience several tumors analogous to human cancers, can act as comparative models for human tumors. ~~These animals experience many naturally-occurring tumors analogous to human cancers. Programs are available to allow companion animals to participate in clinical trials of developmental drugs, with potential benefits to both the animals and the research programs~~ [173].

4.3 Clinical trials

Keeping in mind that a broad-spectrum approach may be used not only by itself, but also as adjuvant therapy with conventional agents, there are numerous potential settings for clinical trials, either for proof of principle or therapeutic goals. Preliminary studies could include metabolomic studies to identify metabolites of dietary interventions, or the pharmacokinetics and pharmacodynamics of phytochemical agents. ~~Studies of high-risk populations, presurgical or neoadjuvant, post-conventional treatments, prevention of recurrence in patients with complete remission, watchful waiting and other non-treatment scenarios, and use in advanced patients or hospice patients can all be contemplated for human trials~~. A variety of settings can be contemplated for clinical trials. One period during which a broad-spectrum approach may be particularly appropriate is the ~~month before and the month after cancer resection~~ perioperative period. Murine data demonstrate that tumor growth accelerates after surgery ~~exploratory laparotomy or other surgical trauma~~; there are also numerous anecdotal reports regarding cancer patients in whom ~~impressive and~~ rapid growth of metastatic tumors has been noted after surgery [174-179]. Further, there is reasonable human evidence that colon or rectal resection results in significant increases in the plasma levels of numerous proangiogenic proteins ~~for 2 to 4 weeks~~ after surgery [180-183]. This period is not generally used for chemotherapy administration because of fears of impaired wound healing, but the above findings provide the rationale and motivation for systemically administering anti-cancer agents perioperatively ~~in the perioperative window~~.

Several non-standard chemotherapy agents, including phytochemicals, have been administered perioperatively in small studies. ~~Immune system up-regulation through non-specific mechanisms has been assessed before and after surgery using a variety of agents such as CpG and fetal liver tyrosine kinase 3 (Flt-3) has been assessed in both murine and small human studies~~ [184-186]. These agents up-regulate immune function via “non-specific” mechanisms. ~~Immunomodulation with H-2 blockers has been associated with improved long term outcome and less immunosuppression in colorectal cancer patients. Phytochemicals or other low-toxicity approaches with anti-cancer effects would therefore be attractive candidates for the perioperative period provided that they do not interfere with wound healing. For example, EGCG and silibinin, an extract of milk thistle, are phytochemicals that meet these criteria. The mechanisms of action of these 2 agents are similar; both interrupt the cell growth cycle, increase apoptosis, and inhibit angiogenesis~~ A Phase I trial assessing the combination of EGCG and silibinin in the setting of colorectal cancer is underway, with both agents given orally before and after surgery. [187-189]. ~~Murine studies have demonstrated that these agents do not significantly impact surgical wound healing~~.Such trials represent an innovative approach to clinical assessment of natural products that can be carried out within a restricted time.

Although clinical trials of phytochemicals and plant extracts in cancer are limited compared to those with conventional chemotherapy, they are by no means lacking. Russo et al. [56] review nearly 50 ongoing and completed trials of phytochemicals and extracts in cancer prevention and therapy, noting that even though clinical research is still limited, preliminary results are promising. Most of the 50 studies took place in the United States, and most included a single phytochemical or single-herb extract. Nearly 3000 controlled trials of Chinese traditional

medicine, 90% concerning herbals, were reviewed by Li et al. [190]. ~~While the problem of inadequate design and reporting of clinical trials is certainly not restricted to herbal medicines [265]~~ Only 16% of traditional medicine trials in this review reported use of adequate methods of randomization, and only a very small percentage reported study blinding, although quality of studies improved through time. ~~It is notable, though, that~~ Most Chinese herbal formulas contain multiple herbs and are aimed at many targets. ~~An abundance of experience with complex formulas is thus represented in Chinese studies~~.

~~However,~~ The design and execution of clinical trials of natural chemicals from plants and foods, however, has been challenging worldwide. An herbal products extension of the CONSORT randomized trial reporting guideline has been published to help improve herbal trial reporting [191]. A review of published studies of *Panax ginseng*, which is common in Chinese formulas but has been studied globally for many conditions, found that only 48% of them reported CONSORT-suggested items, and only 39% reported items from the herbal products extension [192], ~~Indications of improvement of~~ although study designs improved over time ~~were noted for the period of pre-1995 to 2007.~~

~~After appropriate *in vitro* and *in vivo* research, well-designed clinical trials should be a goal of this research model. There are certainly well established processes in place for translational research in cancer, but Given the unique challenges associated with this sort of an approach, we believe there is a priority need for a fundamental change in research strategy, and that nations and cancer foundations should focus their research agendas on the refinement of a broad-spectrum approach. National champions for this agenda are needed. Given the possibility that a highly-effective low cost therapeutic approach could emerge from this process, it is our belief that there are many countries that should be well-positioned to pursue this approach. While nationally sponsored translational research is certainly possible in Europe and North America (where pressures associated with the costs of new therapies continues to be a challenge, as discussed below), countries with well-established herbal medicine traditions and reservoirs, such as China, Japan, Korea, India and others, may be more receptive to translational efforts employing natural products as constituents.~~

4.4 Translational considerations

Assuming that translational research work will involve a substantial combination of therapeutic agents such as those proposed in Table 2 as a starting point, a first step would be the selection of specific targets and approaches for preliminary study. To achieve a truly broad-spectrum effect, one strategy might be to use small doses of every approach that lacks significant contrary interferences. While such a mixture might be made up and applied to cell lines, it could be questioned whether the concentrations that could be achieved in the cells would be physiologically relevant, especially given the low bioavailability of many phytochemicals. In fact, most *in vitro* work on phytochemicals is conducted at concentrations that are not achievable in humans, and the pharmacokinetics and pharmacodynamics of phytochemicals are complex and many are not yet well known, although progress is being made on some agents [193]. Another method to narrow the number of phytochemicals that need to be in an agent might be to select

the phytochemicals that are most widely represented across hallmarks, such as curcumin and resveratrol, and analyze combinations of these agents. Some of the selected approaches, e.g. silibinin, appear to have favorable pharmacokinetics [194]. Other phytochemicals with favorable pharmacokinetics could also be considered for inclusion in a broad-spectrum agent, such as phenethyl isothiocyanate [195]. Research is also urgently needed on the question of the stability of phytochemicals as well as synthetic compounds in mixtures.

Alternative approaches to the question of bioavailability are being explored, especially with the polyphenols. One of the main issues with these compounds, which include quercetin, green tea catechins, curcumin and others, is ensuring that circulating doses of aglycones (one of the active forms of these molecules), are sufficient for activity. After oral supplementation of food-grade molecules at doses safe for humans (200-500 mg/day), only conjugated forms are found in the bloodstream. As an example, quercetin is not found in the plasma as aglycone or as the parent glycosides: at the doses usually employed in intervention studies, it would be found exclusively as methyl, sulfate or glucuronic acid conjugates [196]. This observation discloses a paradox common to many biologically active phytochemicals: if free aglycones are absent *in vivo* after a dietary intake or supplementation with high doses, how can we explain the high biological activity of these molecules, largely described *in vitro*?

Two main hypotheses can be considered. First, conjugated forms of some flavonoids (e.g. quercetin) may be biologically active. Second, after cellular uptake, these metabolites may be de-conjugated, regenerating the free aglycones. To sustain these hypotheses, key issues need to be addressed, such as the efficacy of mechanisms of uptake of polyphenol metabolites and the substrate specificity of each metabolite, which is largely unknown. The use of pure compounds tested *in vitro* may shed light on these questions. Alternatively, pharmacological doses (2-4 g/day) administered orally [197]may saturate the metabolic pathways of conjugation [198]. Efforts are being made, however, to improve bioavailability of these agents, such as microspheres [199], liposomes [200] and nanoparticles [201]. An additional complication is that individuals may vary in their absorption, distribution, metabolism and elimination of phytochemicals, based in some instances on genetic variability [202], dietary habits [203]and potentially on intestinal microbiota [204].

Considerations of quality control ~~of the final product~~ are essential along the spectrum of research from *in vitro* studies to clinical trials. Good agricultural practice, correct botanical identification and good manufacturing practice are mandatory to prevent adulteration, contamination and toxicity [205]. The example of PC-SPES, a botanical cancer remedy that was found to contain indomethacin, warfarin and synthetic estrogens, leading to its withdrawal from the market in 2002 resulted in greater awareness of the need for a strict approach to quality control [206]. ~~The question of whether mixtures of the selected approaches will have adequate stability, or will be subject to degradation due to interactions among various phytochemicals and extracts is likewise of importance. Separating constituents into different dosage forms may be necessary to avoid such interactions.~~

5. Implementation of broad-spectrum research agenda

A variety of practical considerations come into play in translating the proposed research model into a developmental program. These include regulatory considerations, intellectual property, clinical considerations and funding.

5.1 Regulatory considerations

Research on the broad-spectrum model must be undertaken with regulatory constraints in mind. ~~Regarding multi-component natural product therapeutics,~~ Laws controlling herbal medicines, which would likely apply to the broad-spectrum approach we contemplate, vary among countries, but most countries have regulatory paths for herbal or traditional medicine products that differ from those for prescription drugs. ~~Regulatory pathways that could be considered for approval of a multi-component natural product therapeutic vary by country.~~ Regulations relevant to traditional Chinese herbal medicines, perhaps the closest model for the proposed broad-spectrum approach, are reviewed by Fan et al. [207]. A few examples of national regulations regarding herbal medicines, traditional medicines and natural product drugs follow.

The United States has perhaps the most challenging regulations for drug approval, and regulations for mixtures are particularly complex. Some multicomponent formulas, have nevertheless been tested in clinical trials in the US [208,209], but are still being sold only as dietary supplements, without labeling for use in malignancy. The designation of the Botanical Drugs category may offer opportunities to broad-spectrum agents. A recent court decision declaring natural products unpatentable under US law adds an interesting wrinkle to the regulatory framework [210]. In Canada, development as a high-risk Natural Health Product could be considered [211]. China has a variety of regulatory categories that could be used for multicomponent natural product therapeutics [212]. The relevance of Chinese regulations for multi-targeted drugs has been explored [213]. In the European Union, the Marketing Authorization scheme for conventional drugs would need to be used, rather than the Traditional Herbal Regulation Scheme [214], increasing the challenge for developmental research. In India it is likely that New Chemical Entity approval would be required [215], since use in cancer would likely be considered beyond traditional herbal medicine usage. Japan allows herbal medicines to be registered as prescription or over-the-counter drugs [207]; prescription licensing appears likely for an anticancer therapeutic. A variety of regulations exist in other countries, which are beyond the scope of this paper, and which would need to be explored individually. We expect that working under these strict regulations will be difficult, but we do not see it as impossible.

An additional regulatory consideration is the acceptability of the broad-spectrum approach to institutionally-based ethical review boards needed for clinical research. In institutions located in countries in which multi-component herbal formulas are typical of traditional medicine, ethical approval of such formulas is common, as suggested by the large numbers of clinical studies on traditional Chinese herbal medicine [190] and Japanese Kampo medicine [216]. Trials

with multi-component natural products have been conducted under other regulatory schemes as well. For instance, Phase I and Phase Ib studies of BZL101, an extract of *Scutellaria barbata* in metastatic breast cancer have been conducted in the United States [217,218]. A 4-herb combination originating in traditional Chinese medicine, PHY906, has been the subject of a Phase I trial as an adjunct to capecitabine in advanced pancreatic cancer, also in the United States [219]. ~~Individual institutional ethical review boards that have not had prior experience with multi-component or natural product studies may be reluctant to grant approval of clinical studies.~~ In general, provision of sufficient preclinical and drug formulation information, review of prior clinical studies, and possession of appropriate approvals from national-level agencies will facilitate approval of study protocols.

5.2 Intellectual property

~~Another consideration is intellectual property~~. Herbs and natural products in their native forms do not have intellectual property protection, which should help ~~those who are interested~~ in developing ~~the basis for~~ a low-cost, broad-spectrum formulation. Specified extracts and individual phytochemicals may have intellectual property of various types. ~~However, if different research groups undertake research on multi-component natural product therapeutics following this model, it is possible that~~ Researchers could pursue intellectual property protection for specific broad-spectrum therapeutics they develop, as well as licensing to a pharmaceutical company with sufficient resources to support development and testing of the agent. Herbal extracts of some complexity have received patent or trademark status, and have been granted drug approval even in the United States, ~~which is known for its restrictive laws on natural products, in addition to a lack of legal basis for patenting of such products~~. Examples include a mixture of green tea polyphenols known as Polyphenon E and sold as the patented drug Veregen® for genital warts [220], and crofelemer, ~~a mixture of procyanidins and prodelphinidins~~ an extract from the South American plant *Croton lechleri*, approved as the drug Fulyzaq® for HIV-induced diarrhea [221]~~prior to recent court decisions restricting patenting of natural products. These were developed by private companies with standard patent protection. Discussion of~~ The complexities of natural product patenting are beyond the scope of this paper but are covered in depth elsewhere [222].

5.3 Clinical considerations for a multi-component natural product therapeutic

Based on current clinical experience with natural products administered together with conventional drugs, one may anticipate potential concerns with broad-spectrum therapeutics that would be administered jointly with conventional therapies ~~in some clinical models~~. A primary concern ~~with natural products has recently been the topic of drug-supplement~~ interactions between drugs and herbs or phytochemicals, including both pharmacokinetic and pharmacodynamic interactions [223]. This has been of special concern in oncology due to the life-threatening consequences of lowered blood levels of drugs, and the potential for severe side effects when levels of a drug are increased or actions of herbal products reinforce those of conventional agents. ~~Notable in the latter regard is the broad distribution of~~ Antiplatelet

activity is common in natural products [224], and may aggravate clinical consequences in patients with thrombocytopenia due to chemotherapy or other drugs~~, or who receive warfarin treatment~~ [225]. Several other examples of negative interactions are known or suspected. St John's wort (used for depression), contains the strong CYP_{450}3A4 inducer hyperforin, which is known to reduce blood levels of many drugs, including irinotecan [226]. Green tea, which is often taken in high doses by cancer patients, has potential interactions with sunitinib [227], with hepatotoxic drugs [228], and with bortezomib. On the other hand, positive interactions have been observed with green tea and erlotinib, a combination now in clinical trials [229]. Curcumin is one of several natural products that act as chemosensitizers and radiosensitizers for several tumors, while protecting normal tissues [230]. The ability of herbs and other natural products to relieve treatment-related side effects should not be overlooked [231,232].

Furthermore, many natural products possess antioxidant activity. The role of oxidation in cancer progression and treatment is controversial [233]. Oxidative stress is increased in late-stage disease [234], which suggests that suppression would be beneficial. Antioxidants may relieve some adverse treatment effects caused by the reactive oxygen species generated by many chemotherapy drugs, but data on this point are not conclusive [235,236]. Randomized trials of antioxidant supplements given with chemotherapy do not find evidence of reduced efficacy, but research with better study design and larger sample size should be conducted [237]. Additionally, some natural antioxidants, including the polyphenols, manifest pro-oxidant properties in cancer cells, due to interactions with metal ions, which contribute to anticancer effects [238]. This pro-oxidant effect has been hypothesized to underlie the broadly multi-targeted actions of polyphenols such as curcumin and EGCG [239]. However, activity of most chemotherapy drugs depends on generation of ROS which should not be abrogated. Additionally, some oxidative metabolites may act as signaling molecules with anticancer activity [240]. Further, intracellular antioxidants may contribute to drug resistance [241]. Our understanding of the interactions of antioxidants and cancer thus continues to develop [242]. Patients are often warned not to supplement with antioxidants during treatment.

5.4 Funding

Development of new clinical agents that could be approved by ~~developed country~~ regulatory agencies is an expensive endeavor. ~~Calculation of costs for drug development are complex, and different assumptions on the elements of the discovery process to be included in a cost model will result in varying final figures.~~ A recent economic model of drug discovery and development in the United States used industry-appropriate assumptions to estimate that the fully capitalized cost of a typical new single-molecule drug developed is now approximately $1.8 billion, 63% of which is attributable to clinical development (Phase I-III studies) [243]. The details of such estimates are beyond the scope of this paper, but the financial challenges are clear. It is our contention that a multi-component broad-spectrum therapeutic approach is needed to complement and balance the current drug discovery paradigm, which focuses on narrowly scoped approaches and singular molecular targets, including targeted therapies, immunotherapy, "one mouse-one patient" avatars that identify personalized therapeutic regimens by implanting patients' tumors into mice [244]and a variety of other approaches. Such

an approach could be expensive to develop, and could face similar costs for trials and approval. However, a broad-spectrum approach could be aimed at wide applicability among many cancer types and subtypes. Thus, initial investment could be more easily recovered than is the case with narrowly-focused target therapies, since it would have utility across a large group of patients. Whether the development of the broad-spectrum approach should be carried forward by governments, for-profit pharmaceutical companies or even non-profit pharmaceutical companies is an open question.

5.5 Importance for low- and middle-income countries

The possibility that a broad-spectrum approach could be developed that is both effective and inexpensive is an important consideration, ~~since this would have important implications for many countries that simply cannot afford the latest targeted therapies~~, especially in low- and middle-income countries. One of the cost components of drug development is the cost of target identification and validation. However, in the Halifax Project the strategic list of targets that has been developed has been drawn from the open literature, so individual laboratories or nations that are interested in developing a multi-component therapeutic approach can use this information as a starting point (i.e., as a basis for rationally selecting an array of targets).

6. Summary and conclusions

In spite of the importance of targeted therapies now used in treatment and currently in development, it is clear ~~to researchers and clinicians~~ that most cancers cannot be successfully addressed solely with single-target~~, "magic bullet"~~ therapies. The history of cancer treatment ~~since the initiation of the chemotherapy era in the 1950s~~ has taught us the importance of drug resistance, stemming ultimately from genetic heterogeneity in cancers. Our therapeutic tool kit now includes a large array of cytotoxic chemotherapies, molecular target drugs and hormonal therapies. ~~Therapy is becoming increasingly personalized as single- and multiple-gene analyses permit stratification of patients to tailored drugs and regimens.~~ A major paradigm in cancer research, in response to the advances in analysis of the cancer genome, is the development of increasingly targeted therapies, with the hope of reducing toxicity. Examples illustrating the vigor of research and development in this area are several targeted therapies that have received approval in 2013-2014 by the FDA in the United States, including ceritinib [anaplastic lymphokinase (ALK) inhibitor], ramucirumab (VEGFR2 blocker), ibrutinib (tyrosine kinase inhibitor), trametinib (MEK inhibitor) and dabrafenib (B-Raf inhibitor) [245].

At the same time there is an increasing awareness of a need to develop a therapeutic approach to address the genetic heterogeneity within tumors. Even within this group of newly approved agents, the combination of trametinib and dabrafenib was approved for joint use in 2014, due to the rapid (6-7 months) development of resistance to the sole use of B-Raf inhibitors. The emergence of the concept of multiple hallmarks of cancer [24], the nine pathways of progression [34] the listing of 138 driver genes [4] and the recognition of the importance of network pharmacology [49] all attest to the importance of this issue. A recent review similarly

suggests combining anti-inflammatory and antioxidant treatment in long-term maintenance therapy of cancer [246]. It is the contention of the Halifax Project that a broad-spectrum approach to cancer prophylaxis and treatment (i.e., simultaneously attacking many targets) is a strategic and promising response to our increasing understanding of the significance of genetic heterogeneity.

Although current drugs have notably increased initial responsiveness to treatment in comparison to traditional approaches to chemotherapy, there remain situations in which a broad-spectrum approach could make real contributions. Some examples include use as follow-up to conventional ~~adjuvant~~ treatment; for rare cancers ~~and disease stages for which no accepted treatments exist, especially early-stage disease~~; for patients who do not tolerate conventional treatment ~~chemotherapy, hormonal therapy or targeted therapies~~; ~~for patients who experience relapse or progression after targeted treatment;~~ for early-stage disease when aggressive treatment should be avoided; and in hospice and palliative care~~; and for situations in which high-cost agents cannot be obtained~~. If significant interactions with treatments can be avoided, it might even be possible to use such approaches in conjunction with targeted therapies and other treatments. ~~Certainly complementary approaches such as exercise, diet, and biobehavioral therapies are less likely to have negative interactions and may offer both support and a clinical edge in impacting treatment tolerance, response and survival [36]. Intermitting fasting is another example of dietary therapy that could be suggested as an approach for dysregulated metabolism; it may improve response to chemotherapy and radiation in gliomas [305]. although it is only possible intermittently in the clinical setting~~.

What are the implications of this broad-spectrum strategy for current clinical practice? First, clinicians should realize that this paper presents a developmental research program, not clinical guidelines. Use of uninformed selections of phytochemical or botanical extracts in poorly-defined clinical situations is unlikely to deliver positive results. Further, as noted above, concerns with interactions of natural products with conventional treatments should be kept in mind. That said, lifestyle therapies appear to affect multiple molecular targets and to improve the health of cancer patients in a variety of ways [34,148]. Clinical trials are defining beneficial impacts of natural products [247]. The positive implications of dietary therapies for improvement of the metabolic hallmarks of inflammation, deregulated metabolism, genomic instability and immune system evasion should be kept in mind [248,249]. Clinicians choosing to use natural product supplements should attend to product quality and be familiar with advances in the formulation of poorly absorbed polyphenols and other phytochemicals [199-201].

~~The possibility that a broad-spectrum approach could be developed that is both effective and inexpensive is an important consideration, since this would have important implications for many countries that simply cannot afford the latest targeted therapies, especially in low- and middle-income countries. One of the cost components of drug development is the cost of target identification and validation. However, in the Halifax Project the strategic list of targets that has been developed has been drawn from the open literature, so individual laboratories or~~

~~nations that are interested in developing a multi-component therapeutic approach can use this information as a starting point (i.e., as a basis for rationally selecting an array of targets).~~

The development of the broad-spectrum approach is not without cost. A primary need is further development of preclinical models for testing of combinatorial therapies, including study of the stability, pharmacodynamics and pharmacokinetics of agents comprising multiple phytochemicals and other molecules. While some of the targets and approaches recommended in these reviews, are well-known and have been the subject of multiple reviews, others are still only promising leads and may need much better characterization before being adopted as constituents in such an approach. For example, among approaches, curcumin, genistein, resveratrol and EGCG have a wealth of fundamental research, whereas other approaches such as tripterine, oleanoic acid and withaferin A will require additional basic research. Targets are also in need of more basic research, especially in replicative immortality and in deregulated metabolism, a field in which studies of relevant targets are just beginning. The approaches analyzed in these areas are similarly only in the most preliminary stages of research. All the hallmarks, however, include targets and approaches that need substantial basic research. Determining how many of the suggested targets should be included in a broad-spectrum approach is also a question that needs substantial research. Supporting these areas of basic research should be an initial goal of funding efforts.

The pharmacology ~~of lifestyle alterations and~~ of mixtures of natural products is another area in which basic ~~as well as applied~~ research is most relevant to the goals of this project. There is certainly a body of research on complex mixtures of natural products [209,213,216, 217,219]. A recent study suggested that EGCG lowers the concentration of curcumin needed to reduce proliferation and induce apoptosis in uterine leiomyosarcoma cells [250]. Traditional Chinese medicine formulas have also been subjected to extensive pharmacological testing [251,252]. ~~but, as is the case with products from other countries, little has been done to analyze optimal composition of formulas. Some attempts have been made to elucidate the pharmacology and medicinal chemistry of caloric restriction and of a theoretical "epigenetic diet" emphasizing foods containing resveratrol, curcumin, sulforaphane, EGCG and similar phytochemicals [309].~~ However, much remains to be done in quantitative optimization of formulas as well as in selection of optimal natural product extracts or phytochemicals. And although this effort emphasized phytochemicals, it is also important and relevant to study defined botanical and food extracts. Standardized black raspberry extract, for instance, has produced positive results in human trials on apoptosis, angiogenesis and several specific targets selected in the project. [253]. Aged garlic extract [254]increased immunity in advanced cancer patients, and lyophilized strawberries [255] improved premalignant esophageal lesions. Defined herbal extracts such as PHY 906 and BZL101 mentioned above have demonstrated preliminary antitumor activity [218,219]. Stability and pharmacokinetic properties of complex mixtures are another critical research need, as are proper methods of quality control [256]. The development of complex natural product agents appears ripe for cross-disciplinary approaches as well as attention to the process of translational research. Natural products research, in fact, has long been nurtured most successfully in multidisciplinary and collaborative working groups [257], and the teams that authored the reviews in this special issue were notably interdisciplinary themselves.

In view of the challenges as well as the unique opportunities this new concept entails, scientists wishing to take part in the development of broad-spectrum approaches to cancer would do well to commit themselves to a set of new attitudes and skills. ~~Opening minds and laboratories to a wider perspective on pharmacology than has been customary in the era of targeted therapies is likely more of a challenge than it seems at first glance. The process begins with infusion of knowledge on broad-spectrum approaches into graduate and medical school curricula that are already crowded with competing areas of study.~~ Laboratories and grant proposals have achieved success typically based on highly focused exploration of a small intellectual niche. The broad-spectrum approach upends this paradigm. Building linkages with laboratories across campus, or even with the department down the hall, is not always encouraged in academic institutions. But this challenge is not insurmountable, and institutions and granting agencies have successfully mounted efforts that embrace, for instance, natural product development "from the field to the clinic" [258,259]. At the same time, integrative oncology centers globally employ broad-spectrum clinical approaches involving therapies ranging from natural products to meditation in the service of patient needs [260]. There is thus no need to start from absolute zero in building the cross-disciplinary alliances we project will be needed for this effort.

What will be needed is a core group of scientists willing to become advocates for this approach. Advocacy must take place within academic institutions, as institutional silos, perhaps reluctantly, open their doors to collaboration. Institutional review boards and grant offices ~~and institutional press liaisons~~ may need education in the concept of the broad-spectrum approach. Advocacy must take place at higher levels as well. National funding agencies and charitable foundations that currently support cancer research need to heed these recommendations and shift quickly to embrace the rationale for this interdisciplinary team-based approach. Grant review committees may need to confront established interests promoting competing studies with more familiar narrow aims. Creativity in funding initial research efforts will be needed. ~~Researchers may at first access small and more flexible resources for support.~~ International agencies interested in addressing the growth of cancer in low to middle income countries might be convinced that broad-spectrum approaches could result in lower-cost and often more culturally acceptable therapeutic tools for these areas.

Now is the time to begin the work of advocating for broad-spectrum therapeutic approaches in cancer. Scientists need to seize the opportunities provided by the unique information provided in this special issue to expand their acquaintance with this model - and perhaps with the scientists themselves who are already involved in this effort. ~~Work needs to begin in the laboratory, especially in laboratories already accustomed to dealing with natural products, to elaborate and examine more closely the potential for the positive interactions of multiple agents implied in this model of attacking cancer through its multiple hallmarks, driver genes and pathways of progression. In treatment settings, researchers should begin to assess explicitly the impact of behavioral change on a multiplicity of hallmarks, with a view to determining how diet, exercise and mind-body interventions could facilitate this new concept of cancer management.~~ Scientists and clinicians alike should become advocates to their

institutions, to funding sources and to the wider public. ~~through the news media and internet outlets, explaining the need for broad-spectrum approaches that complement the targeted therapies model~~. This dimension of cancer biology and therapy has too much potential to allow it to languish. We look forward to seeing concentrated energy and intellect focused on this new approach, and to seeing it yield significant therapeutic benefits in the future.

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Conflict of Interest Statement

Keith Block is an owner of the Block Center for Integrative Cancer Treatment and of North Shore Nutraceuticals; Charlotte Gyllenhaal is an employee of the Block Center for Integrative Cancer Treatment; Jack Arbiser is the inventor of US Patents involving derivatives of honokiol and NADPH oxidase inhibitors. He has also cofounded ABBY Therapeutics for the development of NADPH oxidase inhibitors; Penny Block is the Executive Director of the Block Center for Integrative Cancer Treatment and President of North Shore Nutraceuticals; Ralph J. DeBerardinis is a member of the scientific advisory boards for Peloton Therapeutics and Agios Pharmaceuticals; Anna Mae E. Diehl has grants from Shire-Research, Metabolon, and Gilead. She is also a consultant for Astrazeneca, Genentech, Japan Tobacco, and the NuSI Foundation; Byoung S. Kwon holds patents for methods regarding anti-CD 137 and adaptive CTL therapeutics; Valter D. Longo has an equity interest in L-Nutra, a company that develops medical food; Kapil Mehta is a scientific advisor to Lifecare Innovations, and holds India Patent 8.765.797, TG2 inhibitors and uses thereof; Michael P. Murphy holds intellectual property in

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Figure Legends

Figure 1. Diagrammatic representation of removal of susceptible cells by a targeted cancer therapy resulting in disease remission, which leaves genetically heterogeneous resistant cells to proliferate, resulting in relapse.

Figure 2. Hallmarks of cancer, sequenced roughly in the order in which these capabilities are acquired by most cancers, as portrayed in the graphical representation of tumor evolution.

Table 1. Prioritized targets with summary of information from cross-validation tables. For each target, the following items are shown: the hallmark(s) for which it was selected, and the number of other hallmarks with which it has complementary relationships, contrary relationships, no known relationships and controversial relationships. For targets that have contrary relationships, the conflicted hallmark(s) are shown. Totals and percentages of each type of relationship are shown at the end of the table.

Target (activity) (hallmark)[1]	Contrary, conflicted hallmarks	Contro-versial	Comple-mentary	None known
Akt (inhbit) (RI)	0	0	10	0
Akt (inhibit) (AP)	0	0	10	0
Akt (inhibit) (TPI))	0	0	10	0
Androgen receptor signaling (suppress) (SPS)	0	2	7	1
AP-1 (inhibit) (TIM)	0	0	7	3
ARID1A (activate) (EAG)	1 TIM	0	4	5
Bcl-2 (inhibit) (AP)	0	1	8	1
CDK 1/2/5/9 (inhibit) (RI)	1 TME	0	8	1
Cell cycle (attenuate) (SPS)	2 IE, TIM	0	8	0
Centrosome clustering (block) (GI)	0	0	7	3
Cholesterol metabolites (inhibit) (TME)	0	0	6	4
Cholesterol synthesis (inhibit) (TME)	0	1	7	2
CKD 4/6 (inhibit) (RI)	1 GI	1	7	1
COX-2 (inhibit) (TPI)	1 AN	0	9	0
CXC chemokine (inhibit) (TPI)	0	3	4	3
Disturbed circadian rhythms (normalize) (AN)	0	2	8	0
DNA damage (prevent) (GI)	1 TPI	3	4	2
DNA repair (enhance) (GI)	1 TPI	3	4	2

E-cadherin (restore) (EAG)	1 AN	3	4	2
E-cadherin (upregulate) (TIM)	1 AN	4	3	2
EF2 (activate) (EAG)	1 TME	0	6	3
EGFR (inhibit) (AP)	0	0	9	1
Elevated insterstitial fluid pressure (reduce) (AN)	0	0	8	2
Endoglin (inhibit) (TME)	0	0	5	5
Endothelial cell migration/tip cell formation (inhibit) (AN)	0	0	6	4
Enox (inhibit) (AP)	0	0	4	6
ER signaling (suppress) (SPS)	0	3	7	0
ER stress (induce) (EAG)	2 AN, TIM	1	6	1
FAK signalling (inhibit) (TIM)	0	0	8	2
Fibrosis (inhibit) (TME)	0	0	5	5
Growth differentiation factor 15 (induce) (EAG)	1 GI	0	4	5
HIF-1 signaling (inhibit) (SPS)	0	0	8	2
Hsp90 (inhibit) (AP)	1 TIM	0	7	2
hTERT (inhibit) (RI)	0	1	7	2
Hypoxia (reduce) (AN)	0	1	9	0
IDO (inhibit) (TME)	0	1	6	3
IGF-1R (inhibit) (EAG)	0	0	8	2
IGFR1 (inhibit) (SPS)	0	0	8	2
IL-2 (induce) (IE)	1 AP	0	4	5
IL-6 (inhibit) (TME)	0	3	7	0
INOS (block) (TPI)	1 AN	1	5	3
JAK (inhibit) (TME)	0	0	9	1
Lymphangiogenesis (impede) (AN)	0	0	4	6

M2 macrophage conversion (inhibit) (TME)	0	0	6	4
Macrophages (activate) (IE)	2 SPS, TIM	2	2	4
Mcl-1 (inhibit) (AP)	0	0	9	1
MIF (block) (TPI)	0	0	8	2
MMP-9 (suppress) (TIM)	0	0	7	3
mTOR (inhibit) (RI)	0	2	7	1
NF-κB signaling (inhibit) (SPS)	0	2	7	1
NF-κB signaling (inhibit) (TIM)	0	2	7	1
NF-κB signaling (inhibit) (TPI)	0	2	7	1
NK cell activity (promote) (IE)	0	0	6	4
NOTCH (block) (EAG)	1 AN	0	7	2
Nuclear exporter CRM1 (inhibit) (AP)	0	0	5	5
PI3K (inhibit) (RI)	0	0	10	0
PI3K/Akt signaling (inhibit) (SPS)	0	0	10	0
PI3K/Akt signaling (inhibit) (TIM)	0	0	10	0
PI3K-Akt (inhibit) (EAG)	0	0	10	0
Poor perfusion (improve) (AN)	0	1	7	2
Proteasome (inhibit) (AP)	0	0	9	1
ROS (inhibit) (TME)	0	2	6	2
Structural abnormalities of vessel walls (inhibit) (AN)	0	0	6	4
Target deficient DNA repair (GI)	1 TPI	2	4	3
Telomerase (inhibit) (GI)	0	0	9	1
Telomerase (inhibit) (RI)	0	0	9	1

TGF-β (inhibit) (TIM)	0	2	7	1
Th1-NK (promote) (IE)	1 TPI	0	4	5
Tight junctions (promote) (TIM)	1 AN	0	5	4
TNF-α (block) (TPI)	1 IE, TIM	1	7	1
Treg lymphocytes (inhibit) (IE)	0	1	5	4
Tumor autophagy (activate) (AP)	1 TPI	4	3	2
Tumor cell metabolism/ acidosis (normalize) (AN)	0	0	8	2
Tumor necrosis (activate) (AP)	2 AN, TME	3	4	1
Tumor-promoting fibroblasts (deactivate) (AN)	0	0	8	2
Tumor-promoting inflammation (suppress) (AN)	0	0	6	4
Urokinase plasminogen activator (suppress) (TIM)	0	0	7	3
VEGF (inhibit) (TME)	0	3	7	0
Wildtype p53 (upregulate) (EAG)	0	0	9	1
Wnt (B-catenin) (inhibit) (SPS)	0	2	7	1
YAP/TEAD activity (inhibit) (EAG)	0	0	5	5
β-catenin/ZEB1 (inactivate) (TIM)	0	0	6	4
γδ T-cell activity (promote) (IE)	2 TPI, AN	0	3	5
Totals	29	65	554	252
%	3.5	7.8	66.7	21.9

[1] AN = Angiogenesis, AP = Resistance to Apoptosis, DM = Deregulated Metabolism, EAG = Evasion of Anti-Growth Signaling, GI = Genetic Instability, IE = Immune Evasion, RI = Replicative Immortality, SPS = Sustained Proliferative Signaling, TIM = Tissue Invasion and Metastasis, TME = Tumor Microenvironment, TPI = Tumor Promoting Inflammation.

Table 2. Prioritized approaches with summary of information from cross-validation tables. For each approach, the following items are shown: the hallmark(s) for which it was selected, and the number of other hallmarks with which it has complementary relationships, contrary relationships, no known relationships and controversial relationships. For approaches that have contrary relationships, the conflicted hallmark(s) are shown. Totals and percentages of each type of relationship are shown at the end of the table. Approaches are natural products except for those noted by asterisks.

Approach (hallmark)*	Contrary, conflicted hallmarks	Contro-versial	Comple-mentary	None known
3-bromopyruvate** (DM)	0	0	0	10
5,6-dihydro-4H-pyrrolo[1,2-b]-pyrrazoles** (TIM)	0	0	1	9
Akt targeted therapies** (AP)	0	1	8	1
Anthocyanins (TPI)	0	0	8	2
Astaxanthin (IE)	0	0	6	4
Astragalus membranaceus (IE)	1 AN	0	5	4
Berberine (TME)	1 IE	0	8	1

BPTES** (DM)	0	0	0	10
Carotenoids (GI)	0	1	9	0
Cordycepin (TIM)	0	1	6	3
Curcumin (AN)	0	0	9	1
Curcumin (EAG)	0	0	9	1
Curcumin (RI)	0	0	10	0
Curcumin (SPS)	0	0	10	0
Curcumin (TME)	0	1	9	0
Curcumin (TPI)	0	0	10	0
Deguelin (EAG)	0	0	6	4
Desoxyrhapontigenin (TME)	0	0	1	9
Dichloroacetate** (DM)	0	0	0	10
Dinacicilib** (RI)	0	0	5	5
EGCG (TPI)	0	0	10	0
EGCG (AN)	0	0	10	0
EGCG (AP)	0	0	10	0
EGCG (EAG)	0	0	10	0
EGCG (RI)	0	0	10	0
EGCG (TME)	0	0	10	0
EGFR targeted therapies** (AP)	0	0	9	1
Eicosapentaenoic acid (TIM)	0	0	7	3
Enterolactone (AN)	0	0	6	4
FX11** (DM)	0	0	0	10
Gamma linolenic acid (TIM)	0	0	6	4
Ganoderic acids (TIM)	0	0	6	4
Ganoderma lucidum (IE)	0	1	7	2
Genistein (EAG)	0	4	6	0
Genistein (RI)	0	5	5	0
Genistein (SPS)	0	4	6	0
Genistein (TME)	0	5	5	0
Genistein (TPI)	0	4	6	0
Gossypol (AP)	0	0	8	2
Grifolin (TIM)	0	0	5	5
Hexachlorophene** (DM)	0	0	0	10
Honokiol (EAG)	0	0	7	3

HS-1793** (IE)	0	0	3	7
Imetelstat** (RI)	0	1	3	6
Isothiocyanate (GI)	0	0	9	1
Kaempferol (AN)	0	0	6	4
Lentinus edodes (IE)	0	0	6	4
Luteolin (EAG)	0	0	8	2
Lycopene (TPI)	0	0	7	3
Melatonin (AN)	0	0	9	1
Naringenin (TME)	0	2	5	3
Oleanoic acid (AN)	0	0	9	1
Onionin A (TME)	0	0	0	10
Pachymic acid (TIM)	0	0	5	5
Palbociclib** (RI)	1 TIM	0	3	6
PARP inhibitor** (GI)	0	0	8	2
Perillyl alcohol (RI)	0	0	9	1
Phenoxodiol** (AP)	0	1	5	4
Piperine (TME)	1 IE	0	6	3
PK15** (DM)	0	0	0	10
Polysaccharide (*Ganoderma lucidum*) (TIM)	0	1	7	2
Resveratrol (AN)	0	1	9	0
Resveratrol (EAG)	0	2	8	0
Resveratrol (GI)	0	2	8	0
Resveratrol (SPS)	0	2	8	0
Resveratrol (TME)	0	2	8	0
Resveratrol (TPI)	0	2	8	0
RO4929097** (EAG)	0	0	8	2
Selenium (GI)	1 TPI	2	5	2
Selinexor** (AP)	0	0	2	8
Silibinin (AN)	0	0	10	0
Silibinin (TIM)	0	0	10	0
TEPP-46** (DM)	0	0	0	10
Trametes versicolor (IE)	0	0	2	8
Tripterine (AN)	0	0	4	6
Triptolide (AP)	1 IE	0	8	1
UMI-77** (AP)	0	0	4	6
Verteporfin** (EAG)	1 AN	0	3	6
Vitamin B (GI)	0	2	2	6
Vitamin D (GI)	0	0	9	1
Withaferin A (AN)	0	0	7	3

β-(1-6)-D-glucan (*A. blazei*) (TIM)	0	0	5	5
Total	7	47	505	261
%	0.9	5.7	61.7	31.8

* AN = Angiogenesis, AP = Resistance to Apoptosis, DM = Deregulated Metabolism, EAG = Evasion of Anti-Growth Signaling, GI = Genetic Instability, IE = Immune Evasion, RI = Replicative Immortality, SPS = Sustained Proliferative Signaling, TIM = Tissue Invasion and Metastasis, TME = Tumor Microenvironment, TPI = Tumor Promoting Inflammation.

** Targeted therapy, synthetic compound or natural product analog/derivative

Table 3. Numbers of targets and therapeutic approaches for each hallmark with the following relationships: complementary relationship, contrary relationship, no known relationship and controversial relationship. Based on cross-validation tables.

Type of relationship	Genetic Instability	Sustained Proliferative Signaling	Tumor-promoting Inflammation	Evasion of Anti-growth Signaling	Resistance to Apoptosis	Replicative Immortality	Deregulated Metabolism	Immune System Evasion	Angiogenesis	Tissue Invasion and Metastasis	Tumor Microenvironment
Targets											
Complementary	30	52	53	53	62	34	55	44	44	65	61
Contrary	2	1	6	0	1	0	0	2	9	5	3
None known	52	24	18	20	13	37	23	34	15	7	9
Controversial	1	5	6	7	4	12	5	4	12	3	7
Therapeutic Approaches											
Complementary	35	51	44	50	62	37	42	22	40	60	64
Contrary	0	0	1	0	0	0	0	3	2	1	0
None known	39	20	26	17	11	37	27	39	23	11	9
Controversial	1	8	5	5	1	1	6	12	7	0	0

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A Broad-Spectrum Integrative Design for Cancer Prevention and Therapy

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Abstract

Targeted therapies and the consequent adoption of “personalized” oncology have achieved notable successes in some cancers, however significant problems remain with this approach. Many targeted therapies are highly toxic, costs are extremely high, and most patients experience relapse after a few disease-free months. Relapses arise from genetic heterogeneity in tumors, which harbor therapy-resistant immortalized cells that have adopted alternate and compensatory pathways (i.e., pathways that are not reliant upon the same mechanisms as those which have been targeted). To address these limitations, an international task force of 177 scientists was assembled to explore the concept of a low-toxicity “broad-spectrum” therapeutic approach that could simultaneously target many key pathways and mechanisms. Using cancer hallmark phenotypes and the tumor microenvironment to account for the various aspects of relevant cancer biology, interdisciplinary teams reviewed each hallmark area and nominated a wide range of high-priority targets (83 in total) that could be modified to improve patient outcomes. For each target, a corresponding low-toxicity therapeutic approach was then suggested; many were phytochemicals. Proposed actions on each target and all of the approaches were further reviewed for known effects on other hallmark areas and the tumor microenvironment. Potential contrary or pro-carcinogenic effects were found for 3.5% of the relationships between targets and other hallmarks, and mixed evidence of complementary and contrary relationships was found for 7.8%. Approximately 67% of the relationships revealed potentially complementary effects, and the remainder had no known relationship. These results suggests that a broad-spectrum approach should be feasible from a safety standpoint. This novel approach has potential to help us address disease relapse, which is a substantial and longstanding problem, so a proposed agenda for future research is offered.

Keywords:

Multi-targeted, cancer hallmarks, phytochemicals, targeted therapy, integrative medicine

Introduction

Cancer is a source of significant and growing mortality worldwide, with an increase to 19.3 million new cancer cases per year projected for 2025. More than half of cancer cases and mortality occur in low- and middle-income countries, and these proportions are expected to increase by 2025 [1]. Current treatments for cancer include surgery, radiotherapy and systemic treatments comprising cytotoxic chemotherapy, hormonal therapy, immunotherapy, and targeted therapies [2]. Cancer continues to stymie clinical treatment efforts, however, and the search for effective therapies continues.

This capstone paper describes the methods and results of a substantial effort by a large international group of biochemical and medical researchers, operating under the name of "The Halifax Project," sponsored by a non-profit organization, Getting To Know Cancer. It summarizes and draws together material from a series of reviews on the hallmarks of cancer, presented in this special issue of Seminars in Cancer Biology, to present a conceptual framework for a new approach to cancer prevention and therapeutics. This approach involves the targeting of many high-priority anti-cancer mechanisms and pathways within a more comprehensive model of treatment and care. We refer to this as a "broad-spectrum" approach (i.e., an approach aimed at a broad spectrum of important mechanisms and pathways). The approach involves combinations of multiple low-toxicity agents that can collectively impact many pathways that are known to be important for genesis and spread of cancer. By making extensive use of chemicals from plants and foods that have already been studied or utilized for cancer prevention and treatment, this approach offers a compelling rationale for addressing the underlying biology of cancer while being efficacious, non-toxic and cost-effective. We come together in the belief that a broad-spectrum approach of this type, in the context of a clinical environment including conventional treatment and attentive to optimal health, would provide real benefit for cancer patients. In this paper we describe the rationale for broad-spectrum therapeutics, detail the methods of the Halifax Project, summarize potential targets and agents related to eleven hallmark features of cancer, propose a research model for the development of broad-spectrum therapies, and call for action to advance this research model.

1. Rationale for Broad-Spectrum Approach

Primary motivations for the development of a broad-spectrum approach stem from the distinct limitations that are evident in many current targeted therapies and the personalized medicine paradigm. Molecular target therapies represent a significant advance in the treatment of cancer. They include drugs such as imatinib, an Abl tyrosine kinase inhibitor that has made chronic myelogenous leukemia a more manageable disease, and inhibitors of vascular endothelial growth factor receptor (VEGFR), such as sunitinib, sorafenib and bevacizumab, used in renal and colon cancers [2]. Other important treatments based on tumor-specific targets are now in use, including examples such as epidermal growth factor receptor (EGFR) inhibitors (gefitinib, erlotinib) used in lung cancer, and the Her2 inhibitor trastuzumab used in breast cancer. Another approach is the synthetic lethal model [3], exemplified by research on poly ADP ribose polymerase (PARP) inhibition, in which mutational loss of one or more redundant

components of a cell survival pathway in tumorigenic cells confers selective sensitivity to drugs that target remaining pathway components.

These drugs target cells bearing one, or at most a few mutated gene products or other abnormalities not found on normal cells. In the therapeutic context, the action of the targeted agents can efficiently address malignant cells, without some of the effects on normal cells notorious in cytotoxic chemotherapy. This enables therapeutic responses and remissions. Over time, however, the genetic heterogeneity of tumors increases, engendering resistance to treatment. Resistant cells drive the emergence of increasingly aggressive disease through clonal expansion and clonal evolution [Figure 1]. Epigenetic modifications may also alter patterns of gene expression Relapses may occur after only a few months, and tumors reappear, sometimes in exactly the same areas in which they originated [4]. Moreover, targeted agents are not without serious side effects, such as treatment-related mortality with bevacizumab and cardiopulmonary arrest with cetuximab. Meta-analysis of trials of recently approved cancer drugs including targeted therapies versus older drugs showed increased rates of grades 3 and 4 toxicity (OR=1.52), treatment discontinuation (OR=1.33) and toxic deaths (OR = 1.40) [5].

[Figure 1 about here]

The efficacy shown to date with targeted therapies, aside from now-established treatments such as bevacizumab and trastuzumab, is nevertheless still limited. Sunitinib, for instance, extends overall survival by 4.6 months in renal cancer, compared with the previous treatment of interferon-α [6]. While statistically significant, this degree of improvement is small comfort to afflicted patients, and challenges the extraordinary monetary investment in drug development as well as costs to the medical system that targeted therapies represent. The MOSCATO 01 trial of molecular triage was able to treat 25 of 111 patients with a variety of advanced cancers using therapies targeted to genomic alterations assessed from tumor biopsies [7]. Of these, 5 patients (20%) experienced partial response and 56% had stable disease. Based on the entire population of 111 patients, this is a partial response of less than 5%, suggesting limited efficacy to date, an outcome also seen in some other studies[8]. On a more hopeful note however, a combination of pertuzumab with trastuzumab and the chemotherapy agent docetaxel was recently found to extend overall survival among the subset of breast cancer patients whose tumors express Her-2 by 15.7 months [9].

Interestingly, harnessing the body´s immune response against the tumor can also result in impressive durable clinical responses, perhaps because the immune system is a paragon of adaptability and can deal with changes in the mutational landscape of cancer to prevent escape from the therapeutic effect. The immunomodulatory antibodies now in advanced clinical trials include ipilimumab (already licensed) as well as nivolimumab and pembrolizumab (licensing anticipated soon) neutralizing two different inhibitory pathways that block anti-tumor T cell responses. These agents have achieved some successes in treating late stage cancers refractory to essentially any other treatments [10]. But even with these agents, response rates are still low and predicting who will respond is a challenge [11,12].

Many of these therapies are somewhat narrowly described as “personalized” because patients’ tumors must be tested for specific mutations to stratify patients to the correct therapy. Viewed in the larger context of individual biological variation, of course, specific mutations drive only the smallest degree of personalization. Truly personalized treatment approaches can be seen to include a much more comprehensive assessment of genetic and even lifestyle factors, such as nutritional, biobehavioral, and exercise habits, along with other host variables such as inflammation and immune status. Such an approach to personalizing treatment can be found in the practice of integrative medicine, which played a significant role in the initial development of this model of broad-spectrum cancer therapy. Some definitions of integrative medicine stress simply the inclusion of complementary and alternative therapies alongside orthodox treatment [13]. A more relevant definition emphasizes a multi-intervention treatment paradigm that utilizes diet, mind-body and physical activity therapies in addition to conventional therapies and dietary supplements [14], based on laboratory testing that enables comprehensive personalization.

The stratification of patients for these targeted and personalized therapies poses practical challenges. As indicated earlier, over 50% of the increase in cancer incidence by 2025 is projected to occur in the developing world [1]. As industrialization develops in lower-income countries, occupational cancers are expected to increase, potentially aggravating this situation [15]. Cancer treatment in many of these countries is already becoming a social-economic challenge due to the expense and medical infrastructure required [16], and the new generation of treatments may further strain local resources. Currently, the platforms used for testing to personalize regimens include whole exome or whole genome sequencing, whole transcriptome sequencing, and comparative genomic hybridization with still others in development. It is likely that such tests, and related expense, will proliferate in the future. Managing treatment toxicity is also a taxing and complex problem, as these toxicities necessitate additional medical interventions.

The expense of the new targeted therapies is also concerning. Eleven of twelve drugs approved by the US Food and Drug Administration in 2012 were priced above $100,000 US per year per patient – perhaps not surprisingly in view of the accelerating costs of drug development [17]. Clinicians have drawn attention to these high costs: in 2013 more than 100 experts in chronic myeloid leukemia coauthored a paper calling for lower prices and broader access to these drugs [18]. The excessive costs have resulted in drugs not being approved for use by national or regional governments where cost-benefit analyses figure in approval processes [19]. While costs are expected to decrease after expiration of patents on the drugs, the costs for treatment in low- or middle-income countries may continue to be problematic. The potential for unsupportable financial stress on health systems challenges the research community to explore other treatment models that can be more sustainable in the face of the worldwide increase in cancer incidence.

The broad-spectrum approach that we describe here is primarily intended to address the two major issues of therapeutic resistance and cost,. It is based on many of the insights of genomic sequencing in cancers. We now know that cancers harbor significant genetic heterogeneity,

even within a single patient [4]. Based on this heterogeneity, cancers routinely evolve resistance to treatment through switching from one growth pathway to another. The proposed strategy employs the basic principles of rational drug design, but aims to stem cancer growth by precisely targeting many growth pathways simultaneously. Some progress is now being made in combining targeted agents so that more than one pathway can be affected, but lack of therapeutic success and significant toxicity and costs make this a challenge [20-23].

We see the broad-spectrum approach as one that is complementary to existing therapies, preferably within the context of a genuinely integrative clinical system. Clinical situations in which such an approach might prove useful include (a) as a follow-up to conventional adjuvant treatment; (b) in situations of rare cancers and disease stages for which no accepted treatments exist; (c) for patients who do not tolerate conventional chemotherapy, hormonal therapy or targeted therapies; (d) for patients who experience relapse or progression after targeted treatment; (e) in hospice or palliative care patients where low- or non-invasive strategies are a legitimate and humane option; and (f) in situations in which high-cost agents cannot be obtained. Because of continuous heterogeneity among cancer cells, and their propensity for genetic instability, even a broad-spectrum approach is unlikely to cause complete remission. However, the design of this approach posed a substantial theoretical challenge, for which we chose to use the hallmarks of cancer as a broad organizing framework.

1.1 Hallmarks of cancer

Douglas Hanahan and Robert A. Weinberg first published their concept of the hallmarks of cancer in 2000 [24]. The hallmarks "constitute an organizing principle that provides a logical framework for understanding the remarkable diversity of neoplastic diseases." This framework encompasses the biological capabilities that cells acquire during the development of cancers that allow them to become malignancies as we know them. Six hallmarks were proposed in the 2000 publication: sustained proliferative signaling, evading growth suppressors, activating invasion and metastasis, enabling replicative immortality, inducing angiogenesis and resisting cell death. The concept of the hallmarks became widely recognized and influential. In 2011, Hanahan and Weinberg expanded on the initial hallmarks to include other areas of cancer biology that they felt were equally important [25]. They pointed out two enabling characteristics critical to the ability of cells to acquire the six hallmarks, and two new hallmark capabilities. They also singled out the crucial nature of the complex tumor microenvironment in the appearance of the cancer phenotype. The enabling characteristics are genomic instability and tumor-promoting inflammation; the new hallmarks are deregulating cellular energetics and avoiding immune destruction.

The hallmarks framework helps to define domains in which high priority targets can be identified for therapeutic targeting. Hanahan and Weinberg point out that agents are in development that target each of the hallmarks. They also note, however, that in response to targeted therapy, cancers may reduce their reliance on a particular hallmark capability, such as angiogenesis, and instead heighten the activity of another capability, such as invasion and metastasis [26]. This reaction has been clinically verified in the case of glioblastoma [27].

Another model, which was proposed by Vogelstein et al. in 2013 [4], also attempts to describe the mechanisms and pathways that are relevant to many cancers. In this model, 12 major signaling pathways that drive cancer growth have been elucidated, including signal transducers and activators of transcription (STAT), NOTCH, DNA damage control and 9 others. These pathways are classified into three cellular processes underlying tumor growth: cell survival, cell fate and genome maintenance. Individual patients with the same cancer can have mutations on different pathways, leading to inter-patient heterogeneity. Yet within each patient there is also substantial heterogeneity, both within each patient's primary tumor, and among and within metastases, with significance for treatment strategies. For instance, the smallest metastases visible through medical imaging may already have thousands of cells that harbor mutations rendering them resistant to current drugs [28].

Cancer mutations, moreover, are not simply a series of isolated targets. Beneath the surface of the cancer genome is a notably complex cellular signaling network, filled with redundancies. The elucidation of rational therapeutic combinations requires dynamic mechanistic models that reach beyond simple targeting [29]. What propels growth, dissemination and thus ineffective treatment and drug resistance actually appears not to be pathways acting in isolation but interconnected, multidirectional and dynamic networks [30]. Even sorafenib, which inhibits multiple kinases, is susceptible to the rapid development of resistance deriving from crosstalk in pathways such as phosphatidylinositide 3-kinase (PI3K)/Akt and Janus kinase (JAK)-STAT, hypoxia-induced signaling or the epithelial-mesenchymal transition [31]. Conventional drug discovery programs are now contemplating systems biology approaches aimed at furthering the network approach to pharmacology. The interdependence of cytokines, chemokines, growth factors, transcription factors, and their resulting proteomes, together with their relevance to cancer prevention and treatment [32], makes systems biology approaches most attractive [33]. This realization makes the significance of a broad-spectrum approach to cancer of even greater importance.

Pharmacologists are not alone in their recognition of the heterogeneity of cancer. A least one clinical center recognizes the significance of this heterogeneity, and intervenes with broad-spectrum approaches to respond to it. In a 2009 book, Life Over Cancer, based on a clinic in operation since 1980, K.I. Block lays out a model of nutraceutical-based targeting of nine "pathways of progression" and six metabolic factors impacting the challenges faced by all cancer patients [34]. The nine growth pathways are proliferation, apoptosis, treatment resistance, immune evasion, angiogenesis, metastasis, cell-to-cell communication, differentiation and immortality. Multiple targeting of these pathways with natural products is used to simultaneous address multiple interconnected growth pathways. Molecular profiling ~~is~~ maps the growth pathways of the individual patient and suggests relevant natural product intervention. The six metabolic "terrain factors" are oxidation, inflammation, glycemia, blood coagulation, immunity and stress chemistry. Terrain-focused interventions are tailored to patients' laboratory test results. Interventions include elimination of maladaptive lifestyle patterns, adjusting exercise habits, improving diet, implementing biobehavioral strategies to diminish adverse consequences of unabated stress/distress, and using natural products and

medications that affect specific targets such as C-reactive protein (CRP) [35], interleukin-6 (IL-6), nuclear factor κ-beta (NF-κB) [36], prostaglandin E2 (PGE2) and leukotriene B4 (LTB4)[37]for inflammation. Clinical observations and literature review suggest potential efficacy for this system in breast cancer (including a near-doubling of survival time of breast cancer patients in integrative care) and potentially other cancers [38,39]. Essentially, Block's clinical model systematically addresses multiple targets and pathways through a specific and selective broad-spectrum approach to treatment. While this system was developed in clinical practice, quite independently from the discussion of hallmarks and enabling characteristics by Hanahan and Weinberg, the conceptual overlap is obvious. That these concepts have already been used in clinical treatment provides powerful support for the viability of a carefully designed broad-spectrum approach.

The model we propose to use to develop a sound framework for a broad-spectrum approach recognizes these broad areas of conceptual overlap and agreement, and can be considered to best align with the hallmarks of cancer framework [24]. For the purposes of this project, we treat the 6 hallmarks, 2 enabling characteristics, 2 emerging hallmarks, and the tumor microenvironment equally as hallmarks of malignancy. From a design standpoint, each of these individual areas encompasses an important aspect of cancer's biology, so each was seen as important to consider for a therapeutic approach aimed at a wide range of high priority targets.

In mid-2012, the framework for this project and approach were shared with Douglas Hanahan. He later independently provided support for this type of approach in a paper, "Rethinking the war on cancer" [40]. Using a military metaphor, he suggests a three-dimensional cancer "battlespace" plan that attacks cancer in a full-scale war rather than individually targeted skirmishes. The first dimension is disruption of cancer's many capabilities, specifically those figuring in the hallmarks. Rather than just removing one capability, as targeted therapies do, he explains that an ideal approach should target all the hallmark capabilities. The second dimension is defense against cancer's armed forces, implying specific targeting of the accessory cell types in the tumor microenvironment, such as tumor-promoting inflammatory cells. The third dimension represents the multiple battlefields of cancer: primary tumor, tumor microenvironment, lymph and blood vessels through which tumors disseminate, draining lymph nodes and distant organs. This dimension suggests still more targets.

A rapidly developing sub-discipline in oncology is the application of genetic and immune analysis of tumor tissue and the concomitant use of personalized therapies and prescriptions. These analyses allow better stratification of patients to treatments and clinical decision-making [41]. In the case of breast cancer alone, tests range from Her-2 testing, the basis of trastuzumab treatment, through Oncotype DX®, a 21-gene panel, to the Symphony™ suite of tests by Agendia which analyzes dozens of genes. These complex analyses assist in treatment decisions based on correlations with clinical outcomes by predicting treatment response, risk of recurrence and outcome. They suggest the size of the network of genes that affect just one cancer, and emphasize the significance of a broad-spectrum attack. Clinical utility of these tests is still under review [42].

The fact remains that, despite impressive progress in genomic and gene expression profiling, it is often impossible to fully characterize the range of immortalized cell variants within any given cancer. The perspectives offered by Hanahan and Block, as well as by the recognition of the network aspects of signaling pathways, however, suggest a larger number of targets may need to be reached. So the 138 driver genes, together with the 12 signaling pathways that comprise them, in addition to the molecular contributors to the hallmarks, and Block's nine pathways of progression and six terrain factors, help us delineate some of the most significant targets that should be taken into account in development of a broad-spectrum approach.

2. Methods

The effort to develop the concept of broad-spectrum targeting of cancer through a complex combination of agents, emphasizing naturally occurring chemicals, was developed by a non-profit organization, Getting To Know Cancer, and implemented within an initiative called "The Halifax Project." The aim of the project was to produce a series of reviews of the cancer hallmarks that could collectively assess and prioritize the many target choices that exist, and also identify non-toxic chemicals (primarily from plants or foods) that could safely be combined to produce an optimized broad-spectrum solution that has both prophylactic and therapeutic potential. To that end, it was envisioned that eleven teams of researchers would produce reviews on the ten cancer hallmarks plus the tumor microenvironment, which was treated as a hallmark for the purposes of this project. Each review was to describe the hallmark, its systemic and cellular dysfunctions, and its relationships to other hallmarks. A priority list of relevant therapeutic targets and corresponding approaches suited to those targets was requested, along with a discussion of research needed in the context of goals of the project. Natural compounds were emphasized because of the growing body of literature that supports the low toxicity and interesting potential that many of these substances have demonstrated (i.e., as targeted therapeutics or in cancer prevention), while recognizing the variable effectiveness of these compounds in human trials as well as the undocumented safety or frank toxicity concerns with many natural products [43].

In recognition of the network of signaling pathways involved not only in drug resistance but the interconnection and maintenance of all the hallmarks, the project implemented a cross-validation step in the evaluation of targets and approaches. Because of the diversity of the targets involved in the 11 hallmark areas, it is not unreasonable to suspect that inhibiting or stimulating a target relevant to one hallmark may have an adverse growth effect or clinically adverse effect on a target in another hallmark. For instance, reducing DNA damage is a potential target for counteracting genomic instability. Activation of the immune system can counter DNA damage by eliminating damaged cells. However, activation of the immune system, while reducing overall levels of DNA damage, can contribute to chronic inflammation. [44].

Similar considerations apply to therapeutic approaches. For instance, triptolide, a component of the Chinese herb *Tripterygium wilfordii*, is known to cause apoptosis in cancer cells [45]. Extracts of the herb have been used in clinical trials for a variety of inflammatory and immune-

linked conditions, and have demonstrated both anti-inflammatory and immune suppressant activity, raising concern for its effect on immune evasion [46,47].

To address this issue, a specially designated cross-validation team was created within the project to evaluate all selected targets and approaches, i.e., to determine whether the inhibition or activation of targets, and the application of approaches, would have negative effects on other hallmarks. Each potential target-hallmark or approach-hallmark interaction was assessed to determine whether the pair had a complementary interaction (i.e., the interaction of the target or approach with the hallmark facilitated anticancer activity), a contrary interaction (i.e., the interaction of the target or approach with the hallmark had a potential adverse tumor-stimulating or tumor-progression effect), a controversial interaction (i.e., mixed indications of anticancer and tumor-stimulating effects), or no known relationship.

It is important to note that the cross-validation team was not given any restrictions for literature selection for this effort, and contributing authors were not restricted to cancer-related research. This approach was taken because it was realized at the outset that this breadth and specificity of knowledge does not yet exist in the literature. As a result, the types and sources of data gathered in this effort varied considerably, although original studies were consistently favored over review articles. Moreover, many studies that were cited in this effort considered only a chemical's ability to instigate or promote an action that mimics a hallmark phenotype in a manner directionally consistent with changes that have been associated with cancer. So while we refer to these as anticancer or tumor-stimulating, the specificity of these activities and their implications for cancer treatment cannot and should not be immediately inferred from this database. In other words, the tabular results from this aspect of the project (Tables 1, 2 and 3) were only compiled to serve as a starting point for future research, rather than a conclusive guide to therapy.

Targets or approaches that have a substantial number of "contrary" assessments are less attractive for inclusion in the broad-spectrum approach. On the other hand, the use of targets and approaches that appear to have the potential for multiple complementary interactions is consistent with principles of rational drug design, and akin to efforts to design "dirty" drugs (a pharmacological term for drugs with multiple targets – as opposed to single targets -- aimed at multidimensional conditions) [48]. Further evaluation of such "dirty" targets and approaches could be undertaken through more specific application of network pharmacology, for which new tools are currently becoming available [49]. The tabulated results, which appear in the individual reviews, are discussed in a later section of this paper.

The review teams needed for the Halifax Project were formed by first circulating an email to a large number of cancer researchers, seeking expressions of their interest in participation. The email was circulated in July 2012 by Getting To Know Cancer, and scientists were encouraged to submit their details on a dedicated webpage that offered additional project detail. From the pool of 703 cancer scientists who responded to the email, 11 team leaders were selected to each lead a group in producing a review of each hallmark, and an additional leader selected for the cross-validation team. Those leaders were then asked to form their own teams (by drawing

from the pool of researchers who expressed interest in the project, and from their own circles of collaborators). Ultimately, 12 teams were formed. Team members were each encouraged to engage a junior researcher as well. This led to fairly large teams but it allowed us to distribute the effort considerably. Team leaders all received project participation guidelines; extensive and ongoing communication from the project leader, Leroy Lowe; copies of the relevant papers of Hanahan and Weinberg; and copies of Life Over Cancer by Block [34] as an example of practical clinical implementation of the broad-spectrum approach. In addition to the two teams, the two guest editors, Anupam Bishayee and Keith Block, were selected for this special issue of *Seminars in Cancer Biology* in which the team reviews are published.

The team leaders and other team members who were able to attend the project workshop met in Halifax, Nova Scotia in August 2013 to discuss the project. Drafts of hallmark team papers were submitted in advance, and summary presentations made at the meeting. Other subject matter presentations included presentations on research funding in the natural products area (Jeffrey D. White, Office of Cancer Complementary and Alternative Medicine, National Cancer Institute) and the concept of driver and passenger genes (Bert Vogelstein, Johns Hopkins). Presentations on integrative cancer therapeutics made at the meeting are summarized below (Keith Block, Penny Block, Block Center for Integrative Cancer Treatment). Group discussions were held to facilitate communication among teams and project staff, and to assist teams in exploring the requirements and rationale for selection of targets and approaches.

Each hallmark team contained the following specialists: a lead author with demonstrated expertise in the hallmark area; domain experts who produced the descriptive review; anticancer phytochemical specialists; oncologists; and support researchers. The cross-validation team conducted background literature searches on the submitted targets and compounds from each review team, verifying their activity in relation to the other hallmarks. This team assessed tradeoffs through determining whether activities of one set of targets and compounds had effects that were complementary to, contrary to, or neutral towards the anticancer activities of each of the other topic areas. Results of the cross-validation effort were tabulated and reviewed by the individual teams. Ambiguous results and areas of disagreement were reconciled, and the tables were ultimately incorporated into each hallmark review.

2.1 Selection of targets and approaches

It was assumed from the outset that, in a translational project aimed at the development of a broad-spectrum approach, there would be a practical upper limit to the number of potential targets in any given cancer that could be targeted. So each hallmark team was asked to select and prioritize up to 10 relevant targets for their hallmark area (bearing in mind that each target would serve as a starting point for the identification of a suitable low-toxicity approach that might be used to reach that target). In theory, it was understood that this could lead to as many as 110 targets for the entire project, and since the teams were also asked to select one therapeutic approach for each target, a maximum of 110 potential therapeutic approaches that may need to be combined.

An “approach” was defined in this project as (1) a technique that will cause the body to respond in a manner that will act on the target (e.g., fasting, exercise etc.), or (2) a procedure involving an entity that can act on the target (e.g., phytochemical, dietary modification, synthetic drug, vaccination with peptides, locally administered oncolytic virus etc). Teams were then asked to identify “favored” approaches with patient safety as a top priority (i.e., least likely to cause harm or side effects even in combination with many other approaches). In addition to safety, other practical considerations for choosing favored approaches were suggested as follows:

- **Efficacy** – Greatest potential to achieve the desired action on the intended target across the widest possible range of cancer types
- **Cost** – Less expensive is better, and by no means cost prohibitive
- **Intellectual Property** – Free of intellectual property constraints if at all possible. Approaches that do not have patents, that cannot be patented, and/or those that have patents that are expired are to be given priority over those that have existing patents.

2.2 Target selection

Extensive discussion took place about the principles of target selection in the context of a broad-spectrum therapeutic approach. Certainly targets that are unique to cancer cells and tumor microenvironments, and that are not known to cause side effects when inhibited pharmacologically, would be a primary consideration. Targets induced by viruses or known carcinogens, would also be major considerations. Consideration of the nature of mutations in the cancer genome and the role of epigenetic modification were also discussed.

It is understood that great effort has been made to sequence the cancer genome to identify the most common mutations seen in different cancers. It is also known that different driver mutations may give rise to variant tumor cells, and the number of driver mutations required is limited, with just 2-8 per patient, which could potentially be assessed through whole genome sequencing of individual cancer patients. However, questions arise about treatment, since most of the currently available drugs are not potent enough to target all susceptible cells. Moreover, the toxicity of existing drugs, if administered in combination protocols, is severely limiting, even at the reduced dosages that may be possible when using multiple agents. A strong rationale supports focusing on low toxicity chemistry (e.g., such as that which has been demonstrated by many anticancer and chemopreventive phytochemicals) as the foundation for a broad-spectrum approach. A number of phytochemicals enhance absorption of other natural products through such mechanisms as cytochrome P450 modification [50], which could also enhance the possibilities for low-toxicity treatment, i.e., by reducing dosages needed for effective treatment.

Many driver genes are actually tumor suppressor genes, and in these cases, it is the loss of the tumor suppressor gene that allows development of cancer. Drugs cannot target these missing genes. Rather they must target unopposed pathways, such as pathways that are active upstream from the missing suppressor gene. For instance, the tumor suppressor forkhead box 0 (FOX0) normally causes apoptosis. If FOX0 is inactivated in cancer, an unopposed pathway

upstream from it is the PI3K/Akt1 signaling pathway, which could alternatively be targeted [51]. The MAP/ERK/MEK pathway, however, can act as a substitute or compensatory pathway to PI3K/Akt1. So, in order to effectively shut down replication, it would seem necessary to address these targets as well.

Cancer-related signaling pathways, including even those that become driver pathways, are also epigenetically modified prior to their genetic modification in cancer pathogenesis [52]. This suggests an emphasis on chemoprevention or treatment of very early cancers. Targeting may be more straightforward to achieve under these conditions, since it is easier to modulate wild-type pathways pharmacologically than to treat the consequences of the onset of widespread aneuploidy. In this case, the cancer phenotype may well precede the cancer genotype by years or more. Combining knowledge of genetic and epigenetic changes in a particular tumor may result in the targeting of key pathways with fewer agents and reduced cost.

A more general consideration is that both direct and indirect targets and approaches can be considered. Direct targets are those that are familiar to us from targeted therapies – oncogenes, tumor suppressor genes, signaling pathways. Indirect approaches, however, are also potentially useful. For instance, evasion of the immune system is a hallmark of cancer [24], and immunomodulatory targets and approaches are appropriate to support the capacities of immune cells to eliminate tumor cells. Immune regulators are, in a sense, inherently multi-targeted due to the complexity of the responses they induce [53]. However, immunity is frequently compromised in patients under treatment with cytotoxic chemotherapies, as well as in the post-surgical period. Consideration of immune system approaches that also support the capacity of patients to tolerate or recover from surgery or toxic therapies indirectly supports the health of cancer patients [54]. The potency of the immune system is illustrated by findings that chemotherapy may enhance anti-tumor immunity if given in the correct sequence, and that cancer refractory to chemotherapy or immune modulation alone may become susceptible to both together [55].

2.3 Approach selection

The need for low-toxicity agents as constituents suggested that phytochemicals –especially those “pre-screened” in humans owing to their presence in foods or traditional medicines -- should be carefully considered during approach selection. Each hallmark team therefore included cancer researchers who had considerable experience working with phytochemicals. In considering phytochemicals and other low-toxicity agents for inclusion in a broad-spectrum approach, however, several limitations in the literature promptly become clear.

First, the level of evidence for the effects of natural products on particular hallmark targets varies widely. The status of laboratory studies and clinical trials on several well-known phytochemicals, e.g. resveratrol, epigallocatechin gallate (EGCG), curcumin, lycopene and others, was recently reviewed [56]. The pleiotropic nature of the effects of these agents on apoptosis and arrest of cell growth has been emphasized, and their potential use in association with chemotherapy drugs has been acknowledged. Novel strategies based on a strategic

combination of phytochemicals with broad-spectrum action together with radiation or chemotherapy agents aimed at overcoming resistance to apoptosis and enhancing sensitivity to treatment are also currently being considered [57,58].

Second, considerable clinical experience with combinations of phytochemicals and other natural agents in treatment of cancer patients exists. Detailed knowledge of the pharmacological effects of combinations of phytochemicals, however, is limited. There is a large literature on herbal combinations used in traditional Chinese medicine in both the laboratory and clinic [59-61], but the quality of older clinical trials is generally low. Additionally, laboratory studies of herbal medicines often use concentrations far higher than are clinically achievable. Supra-physiological concentrations can produce artefactual or irrelevant mechanisms of action or cause toxicity. The limited bioavailability of major phytochemicals makes this especially concerning, although products with improved bioavailability are in development [62]. In general, phytochemical research merits rigorous attention if we hope to gain a more detailed understanding of how these compounds affect the cancer hallmarks. Basic research needs to be followed up with better-designed and statistically-powered clinical trials, if we hope to fully realize the therapeutic potential of phytochemicals.

In addition to laboratory studies and clinical trials, approaches may be suggested by epidemiological studies and the observations of integrative medicine, which uses diet and lifestyle therapies to affect medical conditions including cancer. Observational studies of soy consumption, along with corroborating evidence from clinical studies, suggest that dietary consumption of soy foods consistent with levels in the Japanese diet (2-3 servings daily, containing 25-50mg isoflavones) may be associated with reduced risk of breast cancer incidence and mortality [63]. However, findings from animal studies [64]of negative effects of the soy isoflavone genistein on breast cancer and its treatment suggest that a simplistic reduction of soy to its major phytochemicals is unwarranted – a caution that should be applied to other foods and herbs as well.

At all levels of investigation, the multi-targeted nature of phytochemicals as well as the integrative therapies is notable. Many isolated phytochemicals and herbal may alter large numbers of targets through multifaceted effects on physiology and metabolism [65-67]. A basic complication of these multi-targeted agents, however, is the lack of mechanistic understanding and scientific acceptance of the roles of synergistic or additive molecules in formulation. Although used by human populations for millennia, there remains a question of how to develop and assess multi-component natural product formulations that are suitable for large-scale production. Genome-wide screening for assessment of targeted effects and experimentation with formulation of some herbs typical of traditional Ayurvedic medicine have recently been attempted in Asian laboratories, and are an example of attempts to better understand effects of multi-component agents [68-70].

3. Hallmarks of cancer

In this section we provide brief summaries of each hallmark review included in this special issue of *Seminars in Cancer Biology*. Each summary includes the targets and approaches selected in the hallmark review. Targets and approaches, along with cross-validations, are summarized in Tables 1 and 2. A discussion of the cross-validation results follows. In addition, a summary of the impacts of integrative therapies on cancer-related molecular targets follows the hallmark summary material.

The hallmark summaries are roughly sequenced to capture the acquired capabilities of most cancers (see Figure 2). The section begins with *genomic instability*, an enabling characteristic, followed by *sustained proliferative signaling* and *evasion of anti-growth signaling*, two hallmarks that ensure that proliferation is unabated in cancer cells. These are followed by *resistance to apoptosis* and *replicative immortality*, two layers of defense that are believed to be bypassed in all cancers. Then we discuss *deregulated metabolism and tumor-promoting inflammation*, which signal an important self-reinforcing evolution in the tumor microenvironment. Sections on *angiogenesis* and *tissue invasion and metastasis* speak to disease progression. Finally the *tumor microenvironment* and *immune system evasion* summaries relate to the last lines of defense to be defeated in most cancers.

[Figure 2 about here]

3.1 Genomic instability

Genomic instability plays a critical role in cancer initiation and progression. It provides the means by which a cell or subset of cells acquire a selective advantage over neighboring cells, enabling outgrowth and dominance in the tissue micro-environment. In normal cells, the fidelity of the genome is protected at every stage of the cell cycle by checkpoints. In cancer, the presence of aneuploid cells indicates the failure of one or more of these checkpoints. The resulting genomic heterogeneity may offer the cancer “tissue” growth advantages under selective pressures, including hypoxia, immune- and therapy-related challenges. Understanding these checkpoints, and how they are bypassed in cancer cells, may provide opportunities for the development of rational combinatorial or spectrum treatment strategies, including nutraceuticals such as resveratrol [71,72].

A cell, either transformed or normal, must pass through multiple checkpoints during the process of division. These checkpoints are operated by functional complexes of proteins that either enable the cell to pass through the checkpoint (e.g. proto- or oncogenes) or prevent the progression through the cell cycle (i.e. tumor suppressors). The abundance of these proteins, and their functionality, can be modified by genetic changes to their encoding sequences or by non-genetic, or epigenetic, changes that regulate their abundance. Briefly, small changes to the genes that encode proto-oncogenes or tumor suppressors will positively or negatively impact the function of the gene products. These small changes can be induced by environmental and lifestyle factors, such as toxic substances, diet, and smoking, or they can be encoded in the individual at conception. In the case of DNA damage generated by the environment, it is important that the cell repairs the damage effectively. Dysfunction in the molecules that come

together to recognize and respond to sites of damage is often associated with human cancer. Thus, an understanding of the genetic or epigenetic status of DNA repair genes, and of the nutraceuticals that may modulate them [73], provides an opportunity to predict, detect, prevent and treat a variety of human cancers.

Growing evidences show that vitamins, minerals, and other dietary factors have profound and protective effects against cancer cells, whether they are grown in the lab, in animals, or studied in human populations. In our review, we identify and discuss five priority targets against genomic instability: (1) prevention of DNA damage; (2) enhancement of DNA repair; (3) targeting deficient DNA repair; (4) impairing centrosome clustering; and, (5) inhibition of telomerase activity. Moreover, we highlight vitamin D and B, selenium, carotenoids, PARP inhibitors, resveratrol, and isothiocyanates as priority approaches against genomic instability; these approaches may dampen other enabling characteristics of tumor cells, such as replicative immortality, evasion of anti-growth signaling, tumor promoting inflammation, and oncogenic metabolism [71,74-80].

3.2 Sustained proliferative signaling

Proliferation is an important part of cancer development and progression. This is manifested by altered expression and/or activity of cell cycle related proteins [81,82]. Constitutive activation of many signal transduction pathways also stimulates cell growth. Early steps in tumor development are often associated with a fibrogenic response and development of a hypoxic environment [83,84] which favors the appearance, survival and proliferation of cancer stem cells (CSCs). Part of the survival strategy of CSCs may involve alterations in cell metabolism (such as higher antioxidant levels), and a lack of cell differentiation, which distinguish CSCs from normal tissue stem cells [81,82]. These occur prior to the appearance of tumor, as cells adapt to their changing microenvironment in affected tissue. A part of this adaptation embodies epigenetic and genetic alterations in gene expression [4,85] that also confer resistance to many cytotoxic treatments [86,87]. Thus, adaptive resistance is likely acquired early in the pathogenesis of many tumor types.

Once tumors appear, the continued selection of cells with sustained proliferative signaling further promotes tumor heterogeneity. This is accomplished by growth and metastasis, which may be supported by overproduction of appropriate hormones (in hormonally dependent cancers), by promoting angiogenesis, by undergoing epithelial-to-mesenchymal transition (EMT), by altering the balance between apoptosis, necrosis and autophagy, and by taking cues from surrounding stromal cells. A number of natural compounds (such as EGCG) have been found to inhibit one or more pathways that contribute to proliferation [88-90]. Many of these compounds are nontoxic at doses that inhibit tumor growth and/or prevent the appearance of tumor. However, one of the keys to their efficacy involves their earliest possible therapeutic application. This is because their efficacy is likely to be the greatest in target tissues prior to the appearance of a tumor where cellular heterogeneity is the least. In addition, many of the steps in carcinogenesis prior to tumor appearance are epigenetic in nature, and are more easily targeted by existing compounds, most of which target wild type molecules. This approach limits

adaptive resistance, since early intervention does not have to deal with the issues of aneuploidy, loss of heterozygosity in multiple tumor suppressor genes, and point mutations in oncogenes. The contribution of bioinformatics analyses will be important for identifying signaling pathways and molecular targets that may provide early diagnostic markers and/or critical targets for the development of new drugs or combinations that block tumor formation. Thus, early intervention in pathways and molecules that mediate sustained proliferative signaling will limit adaptive resistance because it targets cells in tissues that have limited genotypic and phenotypic heterogeneity.

Targets selected for sustained proliferative signaling are HIF-1 signaling, NF-κB signaling, PI3K/Akt signaling, Wnt (β-catenin) signaling, IGFR1 signaling, cell cycle (CDKs/cyclins), androgen receptor signaling, and estrogen receptor signaling. Possible therapeutic approaches include curcumin, genistein and resveratrol.

3.3 Evasion of Anti-growth Signaling

Normal cells must acquire the ability to continuously proliferate in order to transform into malignant phenotypes. However, cells have internal programs (anti-growth signaling) to oppose limitless growth. In order to continue to proliferate, cancer cells must somehow evade many anti-growth signals. In general, anti-growth signaling is mediated by the activation of tumor suppressor genes. The Cancer Genome Atlas has compiled data encompassing all tumor types, which indicates that *p53* is the most frequently mutated tumor suppressor gene followed by *PTEN, APC, ATM, BRCA2*, *VHL*, *RB*, *CDKN2A, BRCA1* and *WT1*.

RB1 was the first identified tumor suppressor and deletion of this gene is frequently found in cancers [91]. In many cases, the loss of RB is due to defects in upstream signaling molecules such as inactivation of INK4. Loss of p16ink4a results in unopposed activation of CDK4/6, which phosphorylate the RB protein thereby activating E2F-mediated transcription of genes involved in entry into the cell cycle [92].

Another tumor suppressor frequently deleted due to chromosomal loss is p53 [93]. In fact, more than 50% of all tumors have loss of p53 tumor suppressive functions. Recently, mutant p53 has gained renewed attention due to the fact that along with the loss of tumor suppressive functions, mutant p53 gains oncogenic/tumor promoting functions [94].
Epigenetic silencing of tumor suppressor proteins, which includes DNA methylation, histone methylation and acetylation, is another mechanism through which tumor cells evade anti-growth signaling. Many tumor suppressor genes have been found to have promoter hypermethylation in cancers [95]. Finally, anti-growth signaling plays a major role in treatment response and drug development. For example, the patients with HPV-positive oropharyngeal cancer mostly retain wild-type p53 and have better prognosis and survival.
Although genetic alterations are mostly irreversible, epigenetic repressions are potentially reversible and target for drug development. At least three HDAC inhibitors, belinostat, vorinostat and romidepsin, are currently approved by the U.S. FDA for cancer treatment. Many natural compounds also target the restoration of tumor suppressors through modifying

epigenetic changes [96-100]. Thus, approaches to activate anti-growth signaling will open another chapter for cancer prevention and therapy.

The prioritized targets for anti-growth signaling are the RB, p53, PTEN, Hippo, GDF15, ARID1A, Notch, IGF-1R and others. The approaches are inactivation of E2F by down regulation of pRb using CDK inhibitors, activation of p53 through up-regulation of wild-type p53, activation of PTEN to inhibit PI3K-AKT, activation of Hippo pathways by inhibiting YAP/TEAD activity, induction of GDF15 through p53 activation, activation of ARID1A, blocking NOTCH pathway, and inhibition of IGF-1R to restore tumor suppressor pathways. Furthermore, while the evasion of anti-growth signaling is a critical hallmark of cancer, other hallmarks are similarly important and a more integrative approach is necessary to simultaneously target several hallmarks of cancer to combat this deadly disease.

3.4 Resistance to apoptosis

Apoptosis is a natural way of removing aged and unhealthy cells from the body [101]. However, in cancer, cells lose their ability to undergo apoptosis leading to uncontrolled proliferation and multiplication. These malignant cells are often found to over express many of the proteins that play important roles in resisting the activation of the apoptotic cascade and one of the major hallmarks of human cancers is the intrinsic or acquired resistance to apoptosis [102]. Evasion of apoptosis may contribute to tumor development, progression, and also to treatment resistance, since most of the currently available anticancer therapies including chemotherapy, radio- and immunotherapy primarily act by activating death/apoptotic pathways in cancer cells [103]. Hence, a better understanding of the molecular mechanisms underlying tumor resistance to apoptotic cell death is expected to provide the basis for a rational approach to develop molecular targeted therapies.

Apoptosis resistance is multi-factorial and emanates from the interactions of various molecules and signaling pathways at multiple levels. Several mechanisms exist allowing cells to escape programmed cell death. Among them is the over expression of the anti-apoptotic molecules. The review begins with discussing how B-cell lymphoma-2 (Bcl-2) family proteins play a critical role in the biology of apoptosis resistance. Comprehensive information is presented in regards to the success and challenges in the development of robust agents against the Bcl-2 homology domain 3 (BH3) proteins and how these agents have accelerated toward clinical application. Other cell death mechanisms such as autophagy and necrosis are also discussed and the strategies; in particular, the use of natural agents such EGCG is highlighted. The role of the chaperone protein heat shock protein 70 (Hsp70) in apoptosis resistance is evaluated and suggestions to overcome this critical protein marker using natural products are presented. The article also discusses the molecular mechanisms that support the resistance to apoptosis in different disease models such as glioblastoma, multiple myeloma and chronic lymphocytic leukemia. The role of epigenetic players, particularly the non-coding RNAs/ microRNAs (miRNAs), is also elaborated. The article also touches upon novel targets such as ecto-nicotinamide dinucleotide disulfide thiol exchanger protein (ENOX) and nuclear export protein chromosomal regional maintenance protein 1(CRM1), along with specific strategies to

overcome these important drug resistance promoters. Other targets selected include inhibition of Mcl-1, activation of tumor autophagy, activation of tumor necrosis, inhibition of Hsp90, inhibition of proteasomes, and inhibition of EGFR and Akt. Approaches to these targets include gossypol, UMI-77, EGCG, triptolide, PXD, selinexor, and inhibitors of EGFR and Akt. Collectively, the knowledge gained through greater understanding of the apoptosis resistance targets and specific strategies is anticipated to bring forward a broad form of therapy that could result in better treatment outcome in patients suffering from therapy-resistant cancers.

3.5 Replicative immortality

Replicative immortality, the ability to undergo continuous self-renewal, is necessary for propagation of normal germ cells, but is not a property of normal somatic cells. When acquired by somatic cells that have sustained genetic damage or instability, replicative immortality allows accumulation of sequential aberrations that confer autonomous growth, invasiveness, and therapeutic resistance [104]. As a result, several mechanisms have evolved to regulate replicative potential as a hedge against malignant progression [105]. Senescence, a viable growth arrest characterized by the inability of affected cells to resume proliferation in the presence of appropriate mitogenic factors, is a specific response to the gradual shortening of chromosomal end structures (telomeres) with each round of cell replication, and a more general response to oncogenic and genotoxic stresses. Senescence often involves convergent interdependent activation of tumor suppressors p53 and p16/pRB [106,107], but can still be induced, albeit with reduced sensitivity, when these suppressors are inactivated. Doses of conventional genotoxic drugs required to achieve cancer cell senescence are often much lower than doses required to achieve outright cell death [108]. Additional targeted therapies may induce senescence specifically in cancer cells by blocking cyclin-dependent kinase mediated inhibition of RB-family proteins [109], or by exploiting cancer cells' heightened requirements for maintenance of telomere length through the action of the enzyme telomerase [110]. Developing optimized and truly holistic cancer prevention and treatment regimens will likely incorporate strategies that target replicative immortality.

The chief advantage to be gained by the use of senescence-inducing therapeutic regimens is elimination of the tumor's repopulating ability with reduced collateral damage compared to conventional cytotoxic regimens. There are, however, certain questions and risks associated with this strategy that must be addressed before its clinical adoption. In the case of telomere and telomerase based strategies, replicative senescence may occur more readily in rapidly dividing cancer cells bearing short telomeres than in slowly dividing stem cells with comparatively longer telomeres, but telomere lengths in cancer cells may still be long enough to permit sufficient population doublings for invasion and metastases to occur [110]. Moreover, telomere dysfunction promotes the development of chromosomal instability, which in turn can generate mutations that enable cells to become drug resistant and/or activate alternative lengthening of telomeres (ALT) mechanisms for telomere maintenance and/or become more malignant [111]. High priority should therefore be given to further research into the determinants of senescence stability, as the implications of delayed cell cycle re-entry, permanent cytostasis, or eventual clearance may be profoundly different. Lower doses of

genotoxic drugs needed to induce senescence may reduce collateral damage to critical normal cells, but allow establishment of dormancy and/or adaptive resistance by cancer cells. The microenvironmental and systemic effects of senescent cells also need further clarification, as factors secreted by senescent cells may promote tumorigenic changes in nearby cells. Conversely, since it is almost impossible to kill all the cells in malignant tumors even using the highest tolerated doses of chemotherapy, combined use of an agent that induces or enhances stable senescence in the cancer cells that manage to retain viability might additively or synergistically increase therapeutic efficacy.

A number of potential targets can be singled out for further research, including telomerase, hTERT, mTOR, CDK4/6, CDK 1/2/5/9, Akt and PI3K. Several approaches deserve further research; in particular, the activity of the phytochemicals is still far from clinical utility. These include imetelstat, genistein, perillyl alcohol, palbociclib, dinaciclib, curcumin and EGCG.

3.6 Deregulated metabolism

Deregulated metabolism is a hallmark of cancer, where many cancer cells show increased glucose uptake and produce lactate. This observation is often called the “Warburg effect” [112], but how and why cancer cells reprogram their metabolic state is not well understood. Recent research has focused on understanding the metabolic changes accompanying oncogenesis [24]. A new model of cancer metabolism positions metabolic rewiring in cancer as a coordinated process to support rapid cellular proliferation by tuning cellular energy production needs towards biosynthetic processes. Indeed, several metabolic shifts associated with cancer can be linked to cellular growth, which serve to support biosynthesis of lipids, proteins, nucleic acids required for tumor formation and survival [113].

In several cases, expression of oncogenes and/or loss of tumor suppressors lead directly to changes in metabolism, by expression, activity, or flux of key metabolic nodes. Several components of glucose and glutamine metabolism have emerged as important regulators of metabolism in cancer. In glucose metabolism, hexokinase 2 (HK2), 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (PFKFB3), pyruvate kinase isoform M2 (PKM2) all regulate glycolytic flux. Using a “kitchen sink” analogy for glycolysis, both HK2 and PFKFB3 are regulators of the faucet, and fill up the sink. Conversely, PKM2 regulates the drain. Cancer metabolism turns on the faucet and plugs the drain, which over-spills the glycolytic pathway and provides metabolites used as building blocks for cellular growth. Efforts are underway to identify therapeutic strategies to “turn off the faucet” or “unplug the drain” in glycolysis, limiting cellular growth in cancer. Recent studies have also determined that glutamine is used as a fuel (glutaminolysis) in proliferating cancer cells. Glutamine oxidation can provide carbon and nitrogen for growth, and therefore is an attractive therapeutic target in cancer. Additionally, mutations in genes encoding enzymes directly involved in metabolic pathways have been associated with several types of cancer. Rather than acting as a bystander or facilitator of oncogenesis, aberrant metabolism now has a pro-oncogenic role and has led to the redefinition of some metabolites as ‘oncometabolites’ [114]. Indeed, these

oncometabolites are powerful influencers of proliferation, and are also positioned as new therapeutic targets.

In principle, a broad-spectrum approach to target metabolic shifts in cancer is likely to be a promising therapeutic strategy. However, studies using this approach to target deregulated metabolism in cancer are in their infancy. Lessons could be learned from other strategies to target mitochondria or to target metabolism in order to identify efficacious and safe therapies targeted at cancer metabolism; some drugs targeting metabolism are being re-purposed for their anti-tumorigenic effects. Several approaches have been suggested, including 3-bromopyruvate, PFK-15, TEPP-46, dichloroacetate, hexachlorophene, BPTES and FX11, but data for these must be regarded as extremely preliminary, and they lack sufficient justification to be included in therapy without further study. Most target proteins or pathways identified as having potential to manipulate cancer metabolism have not been directly tested in the context of other hallmarks. The emerging efficacy of physiological interventions that manipulate cancer outcomes, such as fasting, calorie restriction, or exercise, could influence cancer metabolism and other hallmarks of cancer [115]. Future studies directly testing the ability to manipulate deregulated metabolism in cancer will be an important and exciting new area of cancer biology and has potential for treating a variety of cancers.

3.7 Tumor promoting inflammation

Virchow first proposed the role of inflammation in cancer in 1863, while observing the presence of leukocytes in neoplastic , and empirical evidence has since underscored the importance of this linkage [116,117]. The inflammatory milieu promotes a cellular microenvironment that favors the expansion of genomic aberrations and the initiation of carcinogenesis [118]. Chronic inflammation is linked to various phases of tumorigenesis, such as cellular proliferation, transformation, apoptosis evasion, survival, invasion, angiogenesis and metastasis [119-121]. Inflammation is also known to contribute to carcinogenesis through the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which can damage DNA at the site of the tumor [122]. Free radicals and aldehydes, produced during chronic inflammation, can also induce deleterious gene mutation and post-translational modifications of key cancer-related proteins [123].

In addition, chronic inflammation has an influence on immune system constituents that are directly linked with cancer progression. Under normal conditions, immune cells, including macrophages, granulocytes, mast cells, dendritic cells, innate lymphocytes, and natural killer (NK) cells serve as the front line of defense against pathogens. When tissue disruption occurs, macrophages and mast cells secrete matrix-remodeling proteins, cytokines and chemokines, which activate local stromal cells (fibroblasts, adipocytes, vascular cells, etc.) to recruit circulating leukocytes into damaged tissue (acute inflammation), to eliminate pathogens [124]. However, when these processes are initiated in the tumor microenvironment, they are not resolved, which leads to chronic inflammation of the “damaged” (tumor) tissue. Thus, while acute inflammation normally supports and balances two opposing needs for the repair of damaged tissues (apoptosis and wound healing), chronic inflammation represents a loss of this

balance and the resulting confluence of factors has deleterious implications for the immune system [125].

Accordingly, the relationship between tumor-promoting inflammation and cancer is important to consider. So we identified macrophage migration inhibitory factor, cyclooxygenase-2, NF-κB, tumor necrosis factor alpha (TNF-α), inducible nitric oxide synthase, protein kinase B, and chemokines as important anti-inflammatory targets that might be suitable for a multi-pronged therapeutic approach to inflammation suppression. Additionally, we focused on curcumin, resveratrol, EGCG, genistein, lycopene, and anthocyanins, as forms of low-cost chemistry with little to no toxicity that could be employed to reach these targets.

Future translational work should make use of promising agents such as these (combined as constituents within a multi-pronged anti-inflammatory approach) bearing in mind that some of these targets impact the immune system and can increase the risks associated with infection. Bioavailability challenges are also a concern for a number of these agents but recent advances in delivery systems will help address this issue.

3.8 Angiogenesis

Angiogenesis, the expansion of an existing vasculature, is the main mechanism of blood vessel growth in adults, and is therefore essential for tumor development [126].Tumor angiogenesis is switched on by changing the balance between angiogenic factors and inhibitors in favor of angiogenesis [127], a process induced by tumor hypoxia as the tumor grows beyond a size of approximately 1 mm3 [126,128]. At more advanced stages, progressive genomic instability in the tumor leads to mutations in pathways regulating the production of multiple angiogenic factors [129], and stroma cells, also become important sources of sustained angiogenic factor production [130]. These collectively result in a stronger and more complex angiogenic factor profile. It is therefore not surprising that targeted neutralization of a single angiogenic factor, which has been the focus for anti-angiogenic cancer therapy so far, rarely produce long-term, anti-tumor effects [130].

Due to the multifactorial nature of tumor angiogenesis this process is likely to be more efficiently treated by targeting multiple aspects of tumor angiogenesis and vascular dysfunction at the same time. In our review on broad targeting of angiogenesis for cancer prevention and therapy in this issue of Seminars in Cancer Biology, we have identified and discussed 10 of the most important targets for tumor angiogenesis and vascular dysfunction, namely to inhibit endothelial cell migration/tip cell formation, reduce structural abnormalities oft tumor vessels, reduce hypoxia, inhibit lymphangiogenesis, reduce elevated interstitial fluid pressure, reverse poor perfusion normalize disrupted circadian rhythms, suppress tumor promoting inflammation, deactivate tumor promoting fibroblasts and normalize tumor cell metabolism/acidosis.

Currently available non-specific anti-angiogenic agents, able to perform some of these tasks, are however quite toxic, which render them unsuitable for long-term use [129,131,132]. There

is an urgent need to identify alternative compounds that could be used in combination over extended periods of time, targeting tumor angiogenesis broadly and thus lowering the risk of resistance. Plant-derived compounds, phytochemicals, are in many cases better tolerated than the synthetic analogues used in cancer therapy today. Furthermore, they often exhibit broader mechanisms of action and sometimes even higher affinity against important cancer targets compared to the synthetic alternatives [133]. In our review we discuss evidence supporting phytochemicals as anti-angiogenic agents and suggest how these could be combined for maximum effect with minimum toxicity in treatment of cancer. In particular we identify 10 phytochemicals which would be effective as approaches to neutralize the 10 identified targets: oleic acid, tripterine, silibinin, curcumin, EGCG, kaempferol, melatonin, enterolactone, withaferin A and resveratrol. Finally we discuss the optimal use and combination of these phytochemicals in anti-angiogenic therapy focusing on delivery, toxicity and their use in prophylactic regimens.

3.9 Tissue invasion and metastasis

Cancer is a key health issue across the world, causing substantial patient morbidity and mortality. Patient prognosis is tightly linked with metastatic dissemination of the disease to distant sites, with metastatic diseases accounting for a vast percentage of cancer patient mortality [24,134,135]. In order to successfully disseminate to and establish at a secondary location cancer cells must overcome several obstacles as they progress through the metastatic cascade. Successful progression through this cascade is linked with numerous established changes in cellular functions leading to the acquisition of an invasive phenotype. This involves loss of cell-cell contact with the main tumor body, invasion, degradation and migration through surrounding tissue and extra cellular matrix (ECM), secretion of angiogenic / lymphangiogenic factors and intravasation to the blood / lymph vessel, transport around the body and evasion of the immune system, extravasation at the secondary site and establishment of a secondary tumor [136,137].

Hence, factors influencing these processes such as cell adhesion molecules (CAMs), proteolytic matrix degrading enzymes, cell motility and factors involved in the process of EMT have all been subject to scientific scrutiny. Additionally, the complex heterogeneity within tumors, together with cellular interactions between tumor cells and other, non-cancerous, cell types have been established to play key roles in metastatic dissemination and add further complexity to this cascade [135,137]. While advances in the field of cancer research have been made, the process of cancer metastasis and the factors governing cancer spread and establishment at secondary locations are still poorly understood. Current treatment regimes for metastatic disease pose many adverse effects, which can further negatively impact on a subset of patients generally presenting with poorer health conditions. Hence there is a great need to develop new therapeutics that not only target tumor growth and inhibit metastasis but that also have a lower toxicity and reduced inherent side effects. Factors associated with metastasis such disruption of E-cadherin and tight junctions, key signaling pathways, including uPA, PI3K/AKT, FAK, β-catenin/ZEB-1 and TGF-β, together with inactivation of AP-1 and suppression of MMP-9 activity should be considered as key research priorities.

Here, the need is highlighted for new, low toxicity compounds, which interfere with these processes but remain inexpensive alternatives that are readily available and free from intellectual property. Phytochemicals, or natural products, such as those from *Agaricus blazei, Albatrellus confluens, Cordyceps militaris, Ganoderma lucidum, Poria cocos* and *Silybum marianum*, together with diet derived fatty acids gamma linolenic acid (GLA) and eicosapentanoic acid (EPA) and inhibitory compounds have potential to inhibit these key metastatic events. These potential targets and strategies thus present new therapeutic opportunities to both manage cancer metastasis as well as having holistic effect against many of the hallmarks of cancer.

3.10 Tissue interactions in the tumor microenvironment

Cancer arises in the context of an in vivo tumor microenvironment. This microenvironment is a cause and consequence of tumorigenesis that consists of cancer cells and host cells that co-evolve dynamically through indirect and direct cellular interactions, produced metabolites and secreted factors [138,139]. In turn, this environment regulates the ability of a cancer to grow and survive via multiscale effects on many biological programs including cellular proliferation, growth and metabolism, as well as angiogenesis and hypoxia, innate and adaptive immunity [140]. We have identified specific biological programs that could be, based on our most recent understanding, exploited as targets for the prevention and therapy of cancer, including: the inhibition of cholesterol synthesis and metabolites, reactive oxygen species and hypoxia, macrophage activation and conversion, regulation of dendritic cells, regulation of angiogenesis, fibrosis inhibition, endoglin, and cytokine signaling. These programs emerge as examples of important potential nexuses in the regulation of the tumorigenesis and the tumor microenvironment that can be targeted.

The targets we identified include metabolic programs that may broadly influence many cell biology programs that impact tumorigenesis and the tumor microenvironment (cholesterol synthesis and metabolites, reactive oxygen species (ROS) and hypoxia), inflammation, innate and adaptive immunity related programs (macrophage conversion, dendritic cell activation, immune signaling), host microenvironment associated cellular programs (fibrosis, angiogenesis), and cytokine mediated regulatory programs (IL-6, endoglin, and JAK). We particularly focused on identifying approaches for inhibiting these targets that included natural products that have been suggested to have significant anticancer activity. Some of these molecules may more generally influence tumorigenesis and the microenvironment (berberine), others more specifically target reactive oxygen species (ROS; resveratrol, desoxyrhapontigenin) macrophage conversion (onionin A), indoleamine 2,3-dioxygenase (IDO) regulation of dendritic cells (EGCG), cholesterol synthesis (genistein), fibrosis (naringenin), inflammation and immune signaling (piperine) and JAK signaling (zerumbone). We believe that our approach will provide a starting point for examining synergies that might be anticipated in testing certain targets and/or mixtures of natural chemical constituents that may modulate the tumor microenvironment in the treatment and prevention of cancer.

3.11 Immune system evasion

Tumors evade immune attack by several mechanisms including generation of regulatory cells and their secretions, defective antigen presentation, induction of immune suppressive mediators either by cancerous cells themselves or by those in the microenvironment, tolerance, immune deviation and apoptosis.

Current approaches to immune therapy include a) cellular targets, b) molecular targets, c) vaccination therapy, d) therapy by phytochemicals, e) adoptive T cell therapy and f) immunomodulatory antibodies. Of these anti-cancer agents, the most important are those that are targeted in nature and to lesser extent, those that are non-specific in nature. Targeting specific costimulatory molecules such as cytotoxic T-lymphocyte-associated protein-4 (CTLA-4) [141]or programmed cell death protein (PD1/PD-L1) [142]is considered an important anticancer strategy. Of the immunomodulatory antibodies, only anti-CTLA-4 (ipilimumab) has been approved for clinical use in the USA, Canada, United Kingdom, and European Union for melanoma. Also, anti-PD-1 antibodies are showing enormous therapeutic potential in advanced cancers. Targets that are considered appropriate for broad-spectrum, low-toxicity therapeutics are less specific and include enhancing Th1 responses, enhancing γδ T-cells, activation of macrophages, inhibition of Treg lymphocytes, enhancing natural killer cell activity and induction of IL-12.

There are a number of important non-specific anti-cancer agents that have been reported including vaccination therapy, as well as non-specific bacteria-based therapies [143], and phytochemicals [144-146]. Phytochemicals (the biologically active components of fruits and vegetables) have been shown to exert protective effects against cancer. Examples of potential phytochemical approaches include extracts of *Ganoderma lucidum*, *Trametes versicolor*, *Astragalus membranaceus*, and *Lentinus edodes*, as well as astaxanthin and the polyphenol resveratrol analogue HS-1793. There is, however, a downside to phytochemical therapy such as their poor absorption by humans and rapid metabolism and excretion. More work is required to assess which phytochemicals block evasion of immune surveillance and also to determine which phytochemicals promote antitumor responses in cancer patients before these can be recognized for therapeutic value in the clinic.

3.12 Summary of findings on targets and approaches in hallmark reviews

As described above, a cross-validation process was employed to review the proposed actions on each target and all of the approaches for known effects on other hallmark areas and the tumor microenvironment. Anti-carcinogenic synergies and confounding/pro-carcinogenic effects were then compiled and summarized in Tables 1-3. Supplemental tables S1 and S2 contain the aggregated cross-validation tables from each review (with references omitted). More detailed discussion of these interactions can be found in the individual hallmark reviews.

[Tables 1, 2 and 3 about here]

Table 1 shows an alphabetical listing of prioritized targets from each hallmark review, as well as the number of contrary, controversial none known and complementary interactions with all other hallmarks. Note that deregulated metabolism targets do not appear in the table; too little is known about the targets in this new area of research to reliably assess their interactions with other hallmarks. Of these relationships, only 3.5% were contrary, 7.8% were controversial, 21.9% of interaction assessments found no known relationship, and 66.7% were complementary.

Table 2 shows the prioritized therapeutic approaches – the phytochemicals, plant extracts and drugs chosen as modifiers of the priority targets. Of these, 0.9% were contrary, 5.7% were controversial, 31.8% had no known relationships and 61.7% were complementary. Both contrary and controversial interactions indicate potential conflict among the targets and approaches selected for different hallmarks that could result in a broad-spectrum approach with antagonistic, rather than synergistic effects.

The small number of contrary and controversial interactions is encouraging, and suggests that the potential for negative interactions among the selected targets and approach may be limited. However, this may also reflect the common bias in the literature to publish positive antitumor effects. Nearly a third of potential interactions were listed as having no known relationship, suggesting the need for substantially more research in this area. The large number of complementary interactions is also encouraging but may result from indirect or bystander effects as discussed below.

Table 3, in which the different types of interactions of both targets and approaches are listed for each hallmark, also shows some interesting trends. Genetic instability has the largest number of apparent null relationships with the targets and approaches. On the other hand, tumor microenvironment, tissue invasion and metastasis and resistance to apoptosis have the highest number of complementary interactions for both targets and approaches, whereas tumor-promoting inflammation and angiogenesis have the highest number of contrary interactions.

There are a number of limitations that should be noted in this delineation of cross-hallmark relationships. First, the researchers who assembled these results were not asked to distinguish between direct effects on other hallmark areas and reported effects on other hallmark areas that may have resulted in an indirect or “bystander” effect mediated through a different mechanism. In many cases, but not all, this distinction was made. Therefore it is likely that some of the complementary interactions do not represent a fully independent cross-hallmark relationship, but rather are simply indicative of some sort of downstream effect (e.g., within a signaling cascade or via some other signaling molecule that exerts pleiotropic effects). However, we did not feel that this project needed to investigate the nature of these complementary interactions in detail. Instead, our main concern was focused on the possibility that a large number of cross-hallmark relationships might be revealed where actions with pro-carcinogenic or tumor-promoting potential had been reported. It was more important to identify contrary and controversial cross-hallmark interactions than complementary ones, since

targets or approaches that exert pro-carcinogenic actions would normally need to be more carefully assessed (or avoided altogether) in the development of combination approaches or interventions.

The second limitation of these reports of cross-hallmark relationships is related to data quality. In some instances, the underlying evidence used to support the indication of a cross-hallmark relationships was robust, consisting of multiple studies involving detailed *in vitro* and *in vivo* findings. However, in other instances, the underlying evidence that was used to report the existence of a cross-hallmark relationship was quite weak (e.g., consisting of only a single i*n vitro* study involving a single cell-type). Again, the overarching goal in this project was to create a foundation that would allow us to look systematically across the literature in each of these areas, to help us shape the selection of the targets and approaches. So although we realized that not all of these reports of cross-hallmark relationships represented the same level of evidence, we still wanted to examine available evidence to flag targets and approaches where pro-carcinogenic actions had been reported.

There was considerable debate within the task force over the value of tables containing only a simplified indication of a relationship (i.e., + or -) supported by evidence that varied considerably in quality. But since many individual studies and reviews that focus on therapeutic approaches fail to work systematically across the spectrum of incidental actions that might result from combining therapies, it was our opinion that a tabularized framework was the only way to ensure that we had assembled a complete view of cross-hallmark activity.

The types of approaches selected differed among different review teams. While some review teams selected all or mostly phytochemicals or plant extracts, some teams felt that the evidence for these was insufficient, and emphasized other types of molecules, including drugs in development. These may pose more difficulties for translational investigators due to intellectual property, toxicity or other concerns, but may offer advantages in a more clear understanding of their mechanisms. We suggest, however, that the approaches as well as the targets presented in Tables 1 and 2 can be viewed as simply a model for broad-spectrum cancer therapies, rather than as a conclusive or final list. Some of the recommended approaches are clearly experimental, and further research will likely discover compounds, phytochemical or synthetic, that are not on this list that may be useful in a broad-spectrum approach.

Bioavailability of the phytochemicals chosen will also be a concern for future studies. However, the need for development of better preclinical models for screening compounds and testing rationally designed combinatorial therapies composed of compounds from any source is obvious, and should clearly be the first step in the development of the broad-spectrum approach.

3.13 Role of integrative therapies in the broad-spectrum approach

Integrative medicine is an approach to health and healing that "makes use of all appropriate therapeutic approaches, healthcare professionals and disciplines to achieve optimal health and healing" [147]. A comprehensive integrative medicine intervention for cancer patients typically includes nutrition education, mind-body medicine and physical activity components, as well as dietary supplements including herbs, nutraceuticals and phytochemicals [34,148]. Such an intervention may contribute uniquely to a broad-spectrum therapeutic approach through its impact on a wide variety of relevant molecular targets and hallmarks. Hallmarks that may be particularly impacted include genomic instability, tumor-promoting inflammation, deregulated metabolism and immune system evasion. Because of their susceptibility to manipulation by diet, exercise and supplementation, these may be characterized as metabolic hallmarks.

Nutrition has long been the primary focus of research on integrative interventions for cancer. The World Cancer Research Fund and the American Institute for Cancer Research find that diets high in fruits and vegetables substantially reduce risks of several cancers [149]. Cancer prevention diets are also suitable after a cancer diagnosis [150]. For example, colon cancer patients eating a Western diet after diagnosis were at higher risk for recurrence and mortality than those with healthy diets [151]. Breast cancer patients who followed low-fat diets were found to have lost weight and had lower recurrence risks, especially among patients with estrogen receptor-negative cancers [152]. Trials of diets enriched in whole grains, low-glycemic diets, and both low-fat diets and Mediterranean diets enriched in olive oil and almonds reduced levels of inflammation as measured by CRP [153-156]. Low fat diets, weight loss and supplements (anthocyanins and fish oil) have been observed in randomized trials to reduce cytokines and signaling molecules [157-160]. Mind-body interventions have emphasized immune targets, with findings of interventional trials including activation of T-cells and lymphokine-activated killer cells and increased natural killer cell activity [161,162]. Exercise interventions have documented effects on survival, insulin-like growth factor-1, natural killer cell activity, and sex hormones [163-166]. While much work remains to be done on integrative interventions, especially in aiding patients to adopt lifestyle interventions, these preliminary data suggest that integrative medicine may significantly support a broad-spectrum approach to cancer therapy.

4. Proposed research model

The review process for this project has revealed many potential targets and approaches. The cross-validation activity suggests that only a small number of targets and approaches affect other hallmarks in contrary or controversial ways. Indeed the results are quite promising and suggest that the design of a broad-spectrum approach should be feasible from a safety standpoint. Although considerable research will be needed, disease relapse is a substantial and longstanding problem, so this novel model definitely warrants further investigation.

4.1 *In vitro* research

An array of *in vitro* models is available for preliminary study of broad-spectrum formulas. One question is the suitability of receptor-based assays versus cell-based assays. While receptor-based assays may seem more suitable for targeted therapy research, examining the impacts of a putative agent on a molecule such as NF-κB, which is at the intersection of multiple signaling pathways related to inflammation, might be advised. Cultivated cell lines are valuable for preliminary screening of mixtures, but are, in most respects, limited in their predictive ability. Isolated cell lines from clinical samples are an alternative, and use of transformed cancer cells versus non-transformed lines should be discussed. Tissue and organ explants are another useful *in vitro* model.

Basic research on the properties of the natural product and other approaches selected in the reviews needs to continue. The pharmacology of mixtures and combinations of phytochemicals, bioavailability, dose optimization and synergy are among the areas in which research is needed for many phytochemicals [167,168]. However, multicomponent herbal therapies used in traditional and alternative medicine have not received detailed analysis. Network pharmacology could be a means of exploring these presumed synergisms, and efforts are being made to apply this approach to the complex herbal mixtures used in traditional Chinese medicine [169]. Studies on the pharmacokinetics of herbal extracts and phytochemicals, which often begin at the *in vitro* level, are also needed [170].

In sum, given the complexity that is immediately suggested when combinations of approaches are possible, we strongly recommend that well-coordinated, multi-faceted programs be pursued initially to ensure that the constituent approaches that are selected are well-characterized using *in vitro* models, and that delivery methods that are selected for *in vivo* work receive careful evaluation before animal research is undertaken.

4.2 *In vivo* research

Multiple *in vivo* models for further study of broad-spectrum approaches are also available. Two obvious choices are animal tumor models and human tumor xenografts implanted in athymic mice. While human tumor xenografts have the advantage in predicting effects of agents on human cancer cells, animal tumors offer some interesting choices for chemoprevention studies, since several are induced by exposure to various chemicals. The rodent tumors are questionable, however, in their ability to predict human responses to antitumor therapy. Differences in immunity are one consideration, most obviously with athymic mice but also with other animals. Many other differences are known. Rodents and humans, for instance, differ significantly in their blood levels of soy isoflavones after these are administered through a variety of dietary and experimental routes [171]. Isoflavone levels in rodents were 20 to 150 times those in humans, raising questions about the suitability of animals for prediction of phytochemical effects in humans.

Additionally, as shown in different preclinical mouse models, immune and inflammatory responses to cancer differ in young and old individuals, and many cancer treatments are likely to be less effective at older ages. Combination treatment including immunotherapeutic

approaches may be most suitable for older animals. Therefore, there is a strong argument for testing and optimizing combination treatments in suitable model systems before attempting to apply them to cancer patients. The NCI Mouse Models of Human Cancer Consortium [172]has tried to provide the scientific community with accurate, reproducible models of human cancers that can be used in translational and pre-clinical studies. Such improved models could be of great importance for developing combination treatment strategies. Companion animals, such as dogs and cats, which experience several tumors analogous to human cancers, can act as comparative models for human tumors [173].

4.3 Clinical trials

Keeping in mind that a broad-spectrum approach may be used not only by itself, but also as adjuvant therapy with conventional agents, there are numerous potential settings for clinical trials, either for proof of principle or therapeutic goals. Preliminary studies could include metabolomic studies to identify metabolites of dietary interventions, or the pharmacokinetics and pharmacodynamics of phytochemical agents. A variety of settings can be contemplated for clinical trials. One period during which a broad-spectrum approach may be particularly appropriate is the perioperative period. Murine data demonstrate that tumor growth accelerates after surgery; there are also numerous anecdotal reports regarding cancer patients in whom rapid growth of metastatic tumors has been noted after surgery [174-179]. Further, there is reasonable human evidence that colon or rectal resection results in significant increases in the plasma levels of numerous proangiogenic proteins after surgery [180-183]. This period is not generally used for chemotherapy administration because of fears of impaired wound healing, but the above findings provide the rationale and motivation for systemically administering anti-cancer agents perioperatively.

Several non-standard chemotherapy agents, including phytochemicals, have been administered perioperatively in small studies [184-186]. These agents up-regulate immune function via “non-specific” mechanisms. A Phase I trial assessing the combination of EGCG and silibinin in the setting of colorectal cancer is underway, with both agents given orally before and after surgery. [187-189]. Such trials represent an innovative approach to clinical assessment of natural products that can be carried out within a restricted time.

Although clinical trials of phytochemicals and plant extracts in cancer are limited compared to those with conventional chemotherapy, they are by no means lacking. Russo et al. [56] review nearly 50 ongoing and completed trials of phytochemicals and extracts in cancer prevention and therapy, noting that even though clinical research is still limited, preliminary results are promising. Most of the 50 studies took place in the United States, and most included a single phytochemical or single-herb extract. Nearly 3000 controlled trials of Chinese traditional medicine, 90% concerning herbals, were reviewed by Li et al. [190]. ~~[265]~~ Only 16% of traditional medicine trials in this review reported use of adequate methods of randomization, and only a very small percentage reported study blinding, although quality of studies improved through time. Most Chinese herbal formulas contain multiple herbs and are aimed at many targets.

The design and execution of clinical trials of natural chemicals from plants and foods, however, has been challenging worldwide. An herbal products extension of the CONSORT randomized trial reporting guideline has been published to help improve herbal trial reporting [191]. A review of published studies of *Panax ginseng*, which is common in Chinese formulas but has been studied globally for many conditions, found that only 48% of them reported CONSORT-suggested items, and only 39% reported items from the herbal products extension [192], although study designs improved over time.

4.4 Translational considerations

Assuming that translational research work will involve a substantial combination of therapeutic agents such as those proposed in Table 2 as a starting point, a first step would be the selection of specific targets and approaches for preliminary study. To achieve a truly broad-spectrum effect, one strategy might be to use small doses of every approach that lacks significant contrary interferences. While such a mixture might be made up and applied to cell lines, it could be questioned whether the concentrations that could be achieved in the cells would be physiologically relevant, especially given the low bioavailability of many phytochemicals. In fact, most *in vitro* work on phytochemicals is conducted at concentrations that are not achievable in humans, and the pharmacokinetics and pharmacodynamics of phytochemicals are complex and many are not yet well known, although progress is being made on some agents [193]. Another method to narrow the number of phytochemicals that need to be in an agent might be to select the phytochemicals that are most widely represented across hallmarks, such as curcumin and resveratrol, and analyze combinations of these agents. Some of the selected approaches, e.g. silibinin, appear to have favorable pharmacokinetics [194]. Other phytochemicals with favorable pharmacokinetics could also be considered for inclusion in a broad-spectrum agent, such as phenethyl isothiocyanate [195]. Research is also urgently needed on the question of the stability of phytochemicals as well as synthetic compounds in mixtures.

Alternative approaches to the question of bioavailability are being explored, especially with the polyphenols. One of the main issues with these compounds, which include quercetin, green tea catechins, curcumin and others, is ensuring that circulating doses of aglycones (one of the active forms of these molecules), are sufficient for activity. After oral supplementation of food-grade molecules at doses safe for humans (200-500 mg/day), only conjugated forms are found in the bloodstream. As an example, quercetin is not found in the plasma as aglycone or as the parent glycosides: at the doses usually employed in intervention studies, it would be found exclusively as methyl, sulfate or glucuronic acid conjugates [196]. This observation discloses a paradox common to many biologically active phytochemicals: if free aglycones are absent *in vivo* after a dietary intake or supplementation with high doses, how can we explain the high biological activity of these molecules, largely described *in vitro*?

Two main hypotheses can be considered. First, conjugated forms of some flavonoids (e.g. quercetin) may be biologically active. Second, after cellular uptake, these metabolites may be de-conjugated, regenerating the free aglycones. To sustain these hypotheses, key issues need

to be addressed, such as the efficacy of mechanisms of uptake of polyphenol metabolites and the substrate specificity of each metabolite, which is largely unknown. The use of pure compounds tested *in vitro* may shed light on these questions. Alternatively, pharmacological doses (2-4 g/day) administered orally [197] may saturate the metabolic pathways of conjugation [198]. Efforts are being made, however, to improve bioavailability of these agents, such as microspheres [199], liposomes [200] and nanoparticles [201]. An additional complication is that individuals may vary in their absorption, distribution, metabolism and elimination of phytochemicals, based in some instances on genetic variability [202], dietary habits [203]and potentially on intestinal microbiota [204].

Considerations of quality control of the final product are essential along the spectrum of research from *in vitro* studies to clinical trials. Good agricultural practice, correct botanical identification and good manufacturing practice are mandatory to prevent adulteration, contamination and toxicity [205]. The example of PC-SPES, a botanical cancer remedy that was found to contain indomethacin, warfarin and synthetic estrogens, leading to its withdrawal from the market in 2002 resulted in greater awareness of the need for a strict approach to quality control [206].

5. Implementation of broad-spectrum research agenda

A variety of practical considerations come into play in translating the proposed research model into a developmental program. These include regulatory considerations, intellectual property, clinical considerations and funding.

5.1 Regulatory considerations

Research on the broad-spectrum model must be undertaken with regulatory constraints in mind. Laws controlling herbal medicines, which would likely apply to the broad-spectrum approach we contemplate, vary among countries, but most countries have regulatory paths for herbal or traditional medicine products that differ from those for prescription drugs. Regulations relevant to traditional Chinese herbal medicines, perhaps the closest model for the proposed broad-spectrum approach, are reviewed by Fan et al. [207]. A few examples of national regulations regarding herbal medicines, traditional medicines and natural product drugs follow.

The United States has perhaps the most challenging regulations for drug approval, and regulations for mixtures are particularly complex. Some multicomponent formulas, have nevertheless been tested in clinical trials in the US [208,209], but are still being sold only as dietary supplements, without labeling for use in malignancy. The designation of the Botanical Drugs category may offer opportunities to broad-spectrum agents. A recent court decision declaring natural products unpatentable under US law adds an interesting wrinkle to the regulatory framework [210]. In Canada, development as a high-risk Natural Health Product could be considered [211]. China has a variety of regulatory categories that could be used for multicomponent natural product therapeutics [212]. The relevance of Chinese regulations for

multi-targeted drugs has been explored [213]. In the European Union, the Marketing Authorization scheme for conventional drugs would need to be used, rather than the Traditional Herbal Regulation Scheme [214], increasing the challenge for developmental research. In India it is likely that New Chemical Entity approval would be required [215], since use in cancer would likely be considered beyond traditional herbal medicine usage. Japan allows herbal medicines to be registered as prescription or over-the-counter drugs [207]; prescription licensing appears likely for an anticancer therapeutic. A variety of regulations exist in other countries, which are beyond the scope of this paper, and which would need to be explored individually. We expect that working under these strict regulations will be difficult, but we do not see it as impossible.

An additional regulatory consideration is the acceptability of the broad-spectrum approach to institutionally-based ethical review boards needed for clinical research. In institutions located in countries in which multi-component herbal formulas are typical of traditional medicine, ethical approval of such formulas is common, as suggested by the large numbers of clinical studies on traditional Chinese herbal medicine [190] and Japanese Kampo medicine [216]. Trials with multi-component natural products have been conducted under other regulatory schemes as well. For instance, Phase I and Phase Ib studies of BZL101, an extract of *Scutellaria barbata* in metastatic breast cancer have been conducted in the United States [217,218]. A 4-herb combination originating in traditional Chinese medicine, PHY906, has been the subject of a Phase I trial as an adjunct to capecitabine in advanced pancreatic cancer, also in the United States [219]. In general, provision of sufficient preclinical and drug formulation information, review of prior clinical studies, and possession of appropriate approvals from national-level agencies will facilitate approval of study protocols.

5.2 Intellectual property

Herbs and natural products in their native forms do not have intellectual property protection, which should help in developing a low-cost, broad-spectrum formulation. Specified extracts and individual phytochemicals may have intellectual property of various types. Researchers could pursue intellectual property protection for specific broad-spectrum therapeutics they develop, as well as licensing to a pharmaceutical company with sufficient resources to support development and testing of the agent. Herbal extracts of some complexity have received patent or trademark status, and have been granted drug approval even in the United States. Examples include a mixture of green tea polyphenols known as Polyphenon E and sold as the patented drug Veregen® for genital warts [220], and crofelemer, an extract from the South American plant *Croton lechleri*, approved as the drug Fulyzaq® for HIV-induced diarrhea [221] The complexities of natural product patenting are beyond the scope of this paper but are covered in depth elsewhere [222].

5.3 Clinical considerations for a multi-component natural product therapeutic

Based on current clinical experience with natural products administered together with conventional drugs, one may anticipate potential concerns with broad-spectrum therapeutics that would be administered jointly with conventional therapies. A primary concern interactions between drugs and herbs or phytochemicals, including both pharmacokinetic and pharmacodynamic interactions [223]. This has been of special concern in oncology due to the life-threatening consequences of lowered blood levels of drugs, and the potential for severe side effects when levels of a drug are increased or actions of herbal products reinforce those of conventional agents. Antiplatelet activity is common in natural products [224], and may aggravate clinical consequences in patients with thrombocytopenia due to chemotherapy or other drugs [225]. Several other examples of negative interactions are known or suspected. St John's wort (used for depression), contains the strong $CYP_{450}3A4$ inducer hyperforin, which is known to reduce blood levels of many drugs, including irinotecan [226]. Green tea, which is often taken in high doses by cancer patients, has potential interactions with sunitinib [227], with hepatotoxic drugs [228], and with bortezomib. On the other hand, positive interactions have been observed with green tea and erlotinib, a combination now in clinical trials [229]. Curcumin is one of several natural products that act as chemosensitizers and radiosensitizers for several tumors, while protecting normal tissues [230]. The ability of herbs and other natural products to relieve treatment-related side effects should not be overlooked [231,232].

Furthermore, many natural products possess antioxidant activity. The role of oxidation in cancer progression and treatment is controversial [233]. Oxidative stress is increased in late-stage disease [234], which suggests that suppression would be beneficial. Antioxidants may relieve some adverse treatment effects caused by the reactive oxygen species generated by many chemotherapy drugs, but data on this point are not conclusive [235,236]. Randomized trials of antioxidant supplements given with chemotherapy do not find evidence of reduced efficacy, but research with better study design and larger sample size should be conducted [237]. Additionally, some natural antioxidants, including the polyphenols, manifest pro-oxidant properties in cancer cells, due to interactions with metal ions, which contribute to anticancer effects [238]. This pro-oxidant effect has been hypothesized to underlie the broadly multi-targeted actions of polyphenols such as curcumin and EGCG [239]. However, activity of most chemotherapy drugs depends on generation of ROS which should not be abrogated. Additionally, some oxidative metabolites may act as signaling molecules with anticancer activity [240]. Further, intracellular antioxidants may contribute to drug resistance [241]. Our understanding of the interactions of antioxidants and cancer thus continues to develop [242]. Patients are often warned not to supplement with antioxidants during treatment.

5.4 Funding

Development of new clinical agents that could be approved by regulatory agencies is an expensive endeavor. A recent economic model of drug discovery and development in the United States used industry-appropriate assumptions to estimate that the fully capitalized cost of a typical new single-molecule drug developed is now approximately $1.8 billion, 63% of which is attributable to clinical development (Phase I-III studies) [243]. The details of such estimates are beyond the scope of this paper, but the financial challenges are clear. It is our

contention that a multi-component broad-spectrum therapeutic approach is needed to complement and balance the current drug discovery paradigm, which focuses on narrowly scoped approaches and singular molecular targets, including targeted therapies, immunotherapy, “one mouse-one patient” avatars that identify personalized therapeutic regimens by implanting patients’ tumors into mice [244]and a variety of other approaches. Such an approach could be expensive to develop, and could face similar costs for trials and approval. However, a broad-spectrum approach could be aimed at wide applicability among many cancer types and subtypes. Thus, initial investment could be more easily recovered than is the case with narrowly-focused target therapies, since it would have utility across a large group of patients. Whether the development of the broad-spectrum approach should be carried forward by governments, for-profit pharmaceutical companies or even non-profit pharmaceutical companies is an open question.

5.5 Importance for low- and middle-income countries

The possibility that a broad-spectrum approach could be developed that is both effective and inexpensive is an important consideration, especially in low- and middle-income countries. One of the cost components of drug development is the cost of target identification and validation. However, in the Halifax Project the strategic list of targets that has been developed has been drawn from the open literature, so individual laboratories or nations that are interested in developing a multi-component therapeutic approach can use this information as a starting point (i.e., as a basis for rationally selecting an array of targets).

6. Summary and conclusions

In spite of the importance of targeted therapies now used in treatment and currently in development, it is clear that most cancers cannot be successfully addressed solely with single-target therapies. The history of cancer treatment has taught us the importance of drug resistance, stemming ultimately from genetic heterogeneity in cancers. Our therapeutic tool kit now includes a large array of cytotoxic chemotherapies, molecular target drugs and hormonal therapies. A major paradigm in cancer research, in response to the advances in analysis of the cancer genome, is the development of increasingly targeted therapies, with the hope of reducing toxicity. Examples illustrating the vigor of research and development in this area are several targeted therapies that have received approval in 2013-2014 by the FDA in the United States, including ceritinib [anaplastic lymphokinase (ALK) inhibitor], ramucirumab (VEGFR2 blocker), ibrutinib (tyrosine kinase inhibitor), trametinib (MEK inhibitor) and dabrafenib (B-Raf inhibitor) [245].

At the same time there is an increasing awareness of a need to develop a therapeutic approach to address the genetic heterogeneity within tumors. Even within this group of newly approved agents, the combination of trametinib and dabrafenib was approved for joint use in 2014, due to the rapid (6-7 months) development of resistance to the sole use of B-Raf inhibitors. The emergence of the concept of multiple hallmarks of cancer [24], the nine pathways of progression [34] the listing of 138 driver genes [4] and the recognition of the importance of

network pharmacology [49] all attest to the importance of this issue. A recent review similarly suggests combining anti-inflammatory and antioxidant treatment in long-term maintenance therapy of cancer [246]. It is the contention of the Halifax Project that a broad-spectrum approach to cancer prophylaxis and treatment (i.e., simultaneously attacking many targets) is a strategic and promising response to our increasing understanding of the significance of genetic heterogeneity.

Although current drugs have notably increased initial responsiveness to treatment in comparison to traditional approaches to chemotherapy, there remain situations in which a broad-spectrum approach could make real contributions. Some examples include use as follow-up to conventional treatment; for rare cancers; for patients who do not tolerate conventional treatment; for early-stage disease when aggressive treatment should be avoided; and in hospice and palliative care. If significant interactions with treatments can be avoided, it might even be possible to use such approaches in conjunction with targeted therapies and other treatments.

What are the implications of this broad-spectrum strategy for current clinical practice? First, clinicians should realize that this paper presents a developmental research program, not clinical guidelines. Use of uninformed selections of phytochemical or botanical extracts in poorly-defined clinical situations is unlikely to deliver positive results. Further, as noted above, concerns with interactions of natural products with conventional treatments should be kept in mind. That said, lifestyle therapies appear to affect multiple molecular targets and to improve the health of cancer patients in a variety of ways [34,148]. Clinical trials are defining beneficial impacts of natural products [247]. The positive implications of dietary therapies for improvement of the metabolic hallmarks of inflammation, deregulated metabolism, genomic instability and immune system evasion should be kept in mind [248,249]. Clinicians choosing to use natural product supplements should attend to product quality and be familiar with advances in the formulation of poorly absorbed polyphenols and other phytochemicals [199-201].

The development of the broad-spectrum approach is not without cost. A primary need is further development of preclinical models for testing of combinatorial therapies, including study of the stability, pharmacodynamics and pharmacokinetics of agents comprising multiple phytochemicals and other molecules. While some of the targets and approaches recommended in these reviews, are well-known and have been the subject of multiple reviews, others are still only promising leads and may need much better characterization before being adopted as constituents in such an approach. For example, among approaches, curcumin, genistein, resveratrol and EGCG have a wealth of fundamental research, whereas other approaches such as tripterine, oleanoic acid and withaferin A will require additional basic research. Targets are also in need of more basic research, especially in replicative immortality and in deregulated metabolism, a field in which studies of relevant targets are just beginning. The approaches analyzed in these areas are similarly only in the most preliminary stages of research. All the hallmarks, however, include targets and approaches that need substantial basic research. Determining how many of the suggested targets should be included in a broad-spectrum

approach is also a question that needs substantial research. Supporting these areas of basic research should be an initial goal of funding efforts.

The pharmacology of mixtures of natural products is another area in which basic research is most relevant to the goals of this project. There is certainly a body of research on complex mixtures of natural products [209,213,216, 217,219]. A recent study suggested that EGCG lowers the concentration of curcumin needed to reduce proliferation and induce apoptosis in uterine leiomyosarcoma cells [250]. Traditional Chinese medicine formulas have also been subjected to extensive pharmacological testing [251,252]. However, much remains to be done in quantitative optimization of formulas as well as in selection of optimal natural product extracts or phytochemicals. And although this effort emphasized phytochemicals, it is also important and relevant to study defined botanical and food extracts. Standardized black raspberry extract, for instance, has produced positive results in human trials on apoptosis, angiogenesis and several specific targets selected in the project. [253]. Aged garlic extract [254] improved immunity in advanced cancer patients, and lyophilized strawberries [255] improved premalignant esophageal lesions. Defined herbal extracts such as PHY 906 and BZL101 mentioned above have demonstrated preliminary antitumor activity [218,219]. Stability and pharmacokinetic properties of complex mixtures are another critical research need, as are proper methods of quality control [256]. The development of complex natural product agents appears ripe for cross-disciplinary approaches as well as attention to the process of translational research. Natural products research, in fact, has long been nurtured most successfully in multidisciplinary and collaborative working groups [257], and the teams that authored the reviews in this special issue were notably interdisciplinary themselves.

In view of the challenges as well as the unique opportunities this new concept entails, scientists wishing to take part in the development of broad-spectrum approaches to cancer would do well to commit themselves to a set of new attitudes and skills. Laboratories and grant proposals have achieved success typically based on highly focused exploration of a small intellectual niche. The broad-spectrum approach upends this paradigm. Building linkages with laboratories across campus, or even with the department down the hall, is not always encouraged in academic institutions. But this challenge is not insurmountable, and institutions and granting agencies have successfully mounted efforts that embrace, for instance, natural product development “from the field to the clinic” [258,259]. At the same time, integrative oncology centers globally employ broad-spectrum clinical approaches involving therapies ranging from natural products to meditation in the service of patient needs [260]. There is thus no need to start from absolute zero in building the cross-disciplinary alliances we project will be needed for this effort.

What will be needed is a core group of scientists willing to become advocates for this approach. Advocacy must take place within academic institutions, as institutional silos, perhaps reluctantly, open their doors to collaboration. Institutional review boards and grant offices ~~and~~ may need education in the concept of the broad-spectrum approach. Advocacy must take place at higher levels as well. National funding agencies and charitable foundations that currently support cancer research need to heed these recommendations and shift quickly to

embrace the rationale for this interdisciplinary team-based approach. Grant review committees may need to confront established interests promoting competing studies with more familiar narrow aims. Creativity in funding initial research efforts will be needed. International agencies interested in addressing the growth of cancer in low- to middle-income countries might be convinced that broad-spectrum approaches could result in lower-cost and often more culturally acceptable therapeutic tools for these areas.

Now is the time to begin the work of advocating for broad-spectrum therapeutic approaches in cancer. Scientists need to seize the opportunities provided by the unique information provided in this special issue to expand their acquaintance with this model - and perhaps with the scientists themselves who are already involved in this effort. Scientists and clinicians alike should become advocates to their institutions, to funding sources and to the wider public. This dimension of cancer biology and therapy has too much potential to allow it to languish. We look forward to seeing concentrated energy and intellect focused on this new approach, and to seeing it yield significant therapeutic benefits in the future.

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Conflict of Interest Statement

Keith Block is an owner of the Block Center for Integrative Cancer Treatment and of North Shore Nutraceuticals; Charlotte Gyllenhaal is an employee of the Block Center for Integrative Cancer Treatment; Jack Arbiser is the inventor of US Patents involving derivatives of honokiol

and NADPH oxidase inhibitors. He has also cofounded ABBY Therapeutics for the development of NADPH oxidase inhibitors; Penny Block is the Executive Director of the Block Center for Integrative Cancer Treatment and President of North Shore Nutraceuticals; Ralph J. DeBerardinis is a member of the scientific advisory boards for Peloton Therapeutics and Agios Pharmaceuticals; Anna Mae E. Diehl has grants from Shire-Research, Metabolon, and Gilead. She is also a consultant for Astrazeneca, Genentech, Japan Tobacco, and the NuSI Foundation; Byoung S. Kwon holds patents for methods regarding anti-CD 137 and adaptive CTL therapeutics; Valter D. Longo has an equity interest in L-Nutra, a company that develops medical food; Kapil Mehta is a scientific advisor to Lifecare Innovations, and holds India Patent 8.765.797, TG2 inhibitors and uses thereof; Michael P. Murphy holds intellectual property in mitochondrial therapies and has ownership shares in a company called Antipodean Pharmaceuticals Inc. which is trying to commercialize some of these compounds; Jeffrey C. Rathmell received indirect compensation from Novartis while working on this project; Luigi Ricciardiello received an unrestricted research grant from SLA Pharma AG, Switzerland.; John Stagg has a sponsored research agreement with Medimmune LLC; Matthew G. Vander Heiden is a consultant, scientific advisory board member, and owns equity in Agios Pharmaceuticals

Figure Legends

Figure 1. Diagrammatic representation of removal of susceptible cells by a targeted cancer therapy resulting in disease remission, which leaves genetically heterogeneous resistant cells to proliferate, resulting in relapse.

Figure 2. Hallmarks of cancer, sequenced roughly in the order in which these capabilities are acquired by most cancers, as portrayed in the graphical representation of tumor evolution.

Table 1. Prioritized targets with summary of information from cross-validation tables. For each target, the following items are shown: the hallmark(s) for which it was selected, and the number of other hallmarks with which it has complementary relationships, contrary relationships, no known relationships and controversial relationships. For targets that have contrary relationships, the conflicted hallmark(s) are shown. Totals and percentages of each type of relationship are shown at the end of the table.

Target (activity) (hallmark)*	Contrary, conflicted hallmarks	Contro-versial	Comple-mentary	None known
Akt (inhbit) (RI)	0	0	10	0
Akt (inhibit) (AP)	0	0	10	0
Akt (inhibit) (TPI))	0	0	10	0
Androgen receptor signaling (suppress) (SPS)	0	2	7	1
AP-1 (inhibit) (TIM)	0	0	7	3
ARID1A (activate) (EAG)	1 TIM	0	4	5
Bcl-2 (inhibit) (AP)	0	1	8	1
CDK 1/2/5/9 (inhibit) (RI)	1 TME	0	8	1
Cell cycle (attenuate) (SPS)	2 IE, TIM	0	8	0
Centrosome clustering (block) (GI)	0	0	7	3
Cholesterol metabolites (inhibit) (TME)	0	0	6	4
Cholesterol synthesis (inhibit) (TME)	0	1	7	2
CKD 4/6 (inhibit) (RI)	1 GI	1	7	1
COX-2 (inhibit) (TPI)	1 AN	0	9	0
CXC chemokine (inhibit) (TPI)	0	3	4	3
Disturbed circadian rhythms (normalize) (AN)	0	2	8	0
DNA damage (prevent) (GI)	1 TPI	3	4	2
DNA repair (enhance) (GI)	1 TPI	3	4	2

E-cadherin (restore) (EAG)	1 AN	3	4	2
E-cadherin (upregulate) (TIM)	1 AN	4	3	2
EF2 (activate) (EAG)	1 TME	0	6	3
EGFR (inhibit) (AP)	0	0	9	1
Elevated insterstitial fluid pressure (reduce) (AN)	0	0	8	2
Endoglin (inhibit) (TME)	0	0	5	5
Endothelial cell migration/tip cell formation (inhibit) (AN)	0	0	6	4
Enox (inhibit) (AP)	0	0	4	6
ER signaling (suppress) (SPS)	0	3	7	0
ER stress (induce) (EAG)	2 AN, TIM	1	6	1
FAK signalling (inhibit) (TIM)	0	0	8	2
Fibrosis (inhibit) (TME)	0	0	5	5
Growth differentiation factor 15 (induce) (EAG)	1 GI	0	4	5
HIF-1 signaling (inhibit) (SPS)	0	0	8	2
Hsp90 (inhibit) (AP)	1 TIM	0	7	2
hTERT (inhibit) (RI)	0	1	7	2
Hypoxia (reduce) (AN)	0	1	9	0
IDO (inhibit) (TME)	0	1	6	3
IGF-1R (inhibit) (EAG)	0	0	8	2
IGFR1 (inhibit) (SPS)	0	0	8	2
IL-2 (induce) (IE)	1 AP	0	4	5
IL-6 (inhibit) (TME)	0	3	7	0
INOS (block) (TPI)	1 AN	1	5	3
JAK (inhibit) (TME)	0	0	9	1
Lymphangiogenesis (impede) (AN)	0	0	4	6

M2 macrophage conversion (inhibit) (TME)	0	0	6	4
Macrophages (activate) (IE)	2 SPS, TIM	2	2	4
Mcl-1 (inhibit) (AP)	0	0	9	1
MIF (block) (TPI)	0	0	8	2
MMP-9 (suppress) (TIM)	0	0	7	3
mTOR (inhibit) (RI)	0	2	7	1
NF-κB signaling (inhibit) (SPS)	0	2	7	1
NF-κB signaling (inhibit) (TIM)	0	2	7	1
NF-κB signaling (inhibit) (TPI)	0	2	7	1
NK cell activity (promote) (IE)	0	0	6	4
NOTCH (block) (EAG)	1 AN	0	7	2
Nuclear exporter CRM1 (inhibit) (AP)	0	0	5	5
PI3K (inhibit) (RI)	0	0	10	0
PI3K/Akt signaling (inhibit) (SPS)	0	0	10	0
PI3K/Akt signaling (inhibit) (TIM)	0	0	10	0
PI3K-Akt (inhibit) (EAG)	0	0	10	0
Poor perfusion (improve) (AN)	0	1	7	2
Proteasome (inhibit) (AP)	0	0	9	1
ROS (inhibit) (TME)	0	2	6	2
Structural abnormalities of vessel walls (inhibit) (AN)	0	0	6	4
Target deficient DNA repair (GI)	1 TPI	2	4	3
Telomerase (inhibit) (GI)	0	0	9	1
Telomerase (inhibit) (RI)	0	0	9	1

TGF-β (inhibit) (TIM)	0	2	7	1
Th1-NK (promote) (IE)	1 TPI	0	4	5
Tight junctions (promote) (TIM)	1 AN	0	5	4
TNF-α (block) (TPI)	1 IE, TIM	1	7	1
Treg lymphocytes (inhibit) (IE)	0	1	5	4
Tumor autophagy (activate) (AP)	1 TPI	4	3	2
Tumor cell metabolism/ acidosis (normalize) (AN)	0	0	8	2
Tumor necrosis (activate) (AP)	2 AN, TME	3	4	1
Tumor-promoting fibroblasts (deactivate) (AN)	0	0	8	2
Tumor-promoting inflammation (suppress) (AN)	0	0	6	4
Urokinase plasminogen activator (suppress) (TIM)	0	0	7	3
VEGF (inhibit) (TME)	0	3	7	0
Wildtype p53 (upregulate) (EAG)	0	0	9	1
Wnt (B-catenin) (inhibit) (SPS)	0	2	7	1
YAP/TEAD activity (inhibit) (EAG)	0	0	5	5
β-catenin/ZEB1 (inactivate) (TIM)	0	0	6	4
γδ T-cell activity (promote) (IE)	2 TPI, AN	0	3	5
Totals	29	65	554	252
%	3.5	7.8	66.7	21.9

* AN = Angiogenesis, AP = Resistance to Apoptosis, DM = Deregulated Metabolism, EAG = Evasion of Anti-Growth Signaling, GI = Genetic Instability, IE = Immune Evasion, RI = Replicative Immortality, SPS = Sustained Proliferative Signaling, TIM = Tissue Invasion and Metastasis, TME = Tumor Microenvironment, TPI = Tumor Promoting Inflammation.

Table 2. Prioritized approaches with summary of information from cross-validation tables. For each approach, the following items are shown: the hallmark(s) for which it was selected, and the number of other hallmarks with which it has complementary relationships, contrary relationships, no known relationships and controversial relationships. For approaches that have contrary relationships, the conflicted hallmark(s) are shown. Totals and percentages of each type of relationship are shown at the end of the table. Approaches are natural products except for those noted by asterisks.

Approach (hallmark)*	Contrary, conflicted hallmarks	Contro-versial	Comple-mentary	None known
3-bromopyruvate** (DM)	0	0	0	10
5,6-dihydro-4H-pyrrolo[1,2-b]-pyrrazoles** (TIM)	0	0	1	9
Akt targeted therapies** (AP)	0	1	8	1
Anthocyanins (TPI)	0	0	8	2
Astaxanthin (IE)	0	0	6	4
Astragalus membranaceus (IE)	1 AN	0	5	4
Berberine (TME)	1 IE	0	8	1

BPTES** (DM)	0	0	0	10
Carotenoids (GI)	0	1	9	0
Cordycepin (TIM)	0	1	6	3
Curcumin (AN)	0	0	9	1
Curcumin (EAG)	0	0	9	1
Curcumin (RI)	0	0	10	0
Curcumin (SPS)	0	0	10	0
Curcumin (TME)	0	1	9	0
Curcumin (TPI)	0	0	10	0
Deguelin (EAG)	0	0	6	4
Desoxyrhapontigenin (TME)	0	0	1	9
Dichloroacetate** (DM)	0	0	0	10
Dinacicilib** (RI)	0	0	5	5
EGCG (TPI)	0	0	10	0
EGCG (AN)	0	0	10	0
EGCG (AP)	0	0	10	0
EGCG (EAG)	0	0	10	0
EGCG (RI)	0	0	10	0
EGCG (TME)	0	0	10	0
EGFR targeted therapies** (AP)	0	0	9	1
Eicosapentaenoic acid (TIM)	0	0	7	3
Enterolactone (AN)	0	0	6	4
FX11** (DM)	0	0	0	10
Gamma linolenic acid (TIM)	0	0	6	4
Ganoderic acids (TIM)	0	0	6	4
Ganoderma lucidum (IE)	0	1	7	2
Genistein (EAG)	0	4	6	0
Genistein (RI)	0	5	5	0
Genistein (SPS)	0	4	6	0
Genistein (TME)	0	5	5	0
Genistein (TPI)	0	4	6	0
Gossypol (AP)	0	0	8	2
Grifolin (TIM)	0	0	5	5
Hexachlorophene** (DM)	0	0	0	10
Honokiol (EAG)	0	0	7	3

HS-1793** (IE)	0	0	3	7
Imetelstat** (RI)	0	1	3	6
Isothiocyanate (GI)	0	0	9	1
Kaempferol (AN)	0	0	6	4
Lentinus edodes (IE)	0	0	6	4
Luteolin (EAG)	0	0	8	2
Lycopene (TPI)	0	0	7	3
Melatonin (AN)	0	0	9	1
Naringenin (TME)	0	2	5	3
Oleanoic acid (AN)	0	0	9	1
Onionin A (TME)	0	0	0	10
Pachymic acid (TIM)	0	0	5	5
Palbociclib** (RI)	1 TIM	0	3	6
PARP inhibitor** (GI)	0	0	8	2
Perillyl alcohol (RI)	0	0	9	1
Phenoxodiol** (AP)	0	1	5	4
Piperine (TME)	1 IE	0	6	3
PK15** (DM)	0	0	0	10
Polysaccharide (*Ganoderma lucidum*) (TIM)	0	1	7	2
Resveratrol (AN)	0	1	9	0
Resveratrol (EAG)	0	2	8	0
Resveratrol (GI)	0	2	8	0
Resveratrol (SPS)	0	2	8	0
Resveratrol (TME)	0	2	8	0
Resveratrol (TPI)	0	2	8	0
RO4929097** (EAG)	0	0	8	2
Selenium (GI)	1 TPI	2	5	2
Selinexor** (AP)	0	0	2	8
Silibinin (AN)	0	0	10	0
Silibinin (TIM)	0	0	10	0
TEPP-46** (DM)	0	0	0	10
Trametes versicolor (IE)	0	0	2	8
Tripterine (AN)	0	0	4	6
Triptolide (AP)	1 IE	0	8	1
UMI-77** (AP)	0	0	4	6
Verteporfin** (EAG)	1 AN	0	3	6
Vitamin B (GI)	0	2	2	6
Vitamin D (GI)	0	0	9	1
Withaferin A (AN)	0	0	7	3

β-(1-6)-D-glucan (*A. blazei*) (TIM)	0	0	5	5
Total	7	47	505	261
%	0.9	5.7	61.7	31.8

* AN = Angiogenesis, AP = Resistance to Apoptosis, DM = Deregulated Metabolism, EAG = Evasion of Anti-Growth Signaling, GI = Genetic Instability, IE = Immune Evasion, RI = Replicative Immortality, SPS = Sustained Proliferative Signaling, TIM = Tissue Invasion and Metastasis, TME = Tumor Microenvironment, TPI = Tumor Promoting Inflammation.

** Targeted therapy, synthetic compound or natural product analog/derivative

Table 3. Numbers of targets and therapeutic approaches for each hallmark with the following relationships: complementary relationship, contrary relationship, no known relationship and controversial relationship. Based on cross-validation tables.

Type of relationship	Genetic Instability	Sustained Proliferative Signaling	Tumor-promoting Inflammation	Evasion of Anti-growth Signaling	Resistance to Apoptosis	Replicative Immortality	Deregulated Metabolism	Immune System Evasion	Angiogenesis	Tissue Invasion and Metastasis	Tumor Microenvironment
Targets											
Complementary	30	52	53	53	62	34	55	44	44	65	61
Contrary	2	1	6	0	1	0	0	2	9	5	3
None known	52	24	18	20	13	37	23	34	15	7	9
Controversial	1	5	6	7	4	12	5	4	12	3	7
Therapeutic Approaches											
Complementary	35	51	44	50	62	37	42	22	40	60	64
Contrary	0	0	1	0	0	0	0	3	2	1	0
None known	39	20	26	17	11	37	27	39	23	11	9
Controversial	1	8	5	5	1	1	6	12	7	0	0

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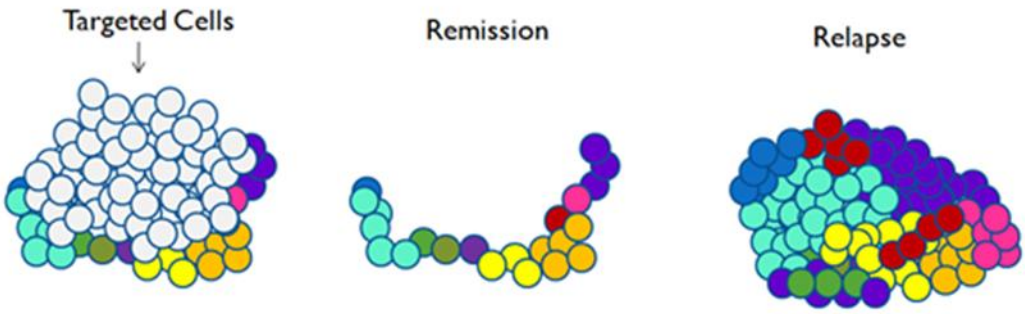
Figure



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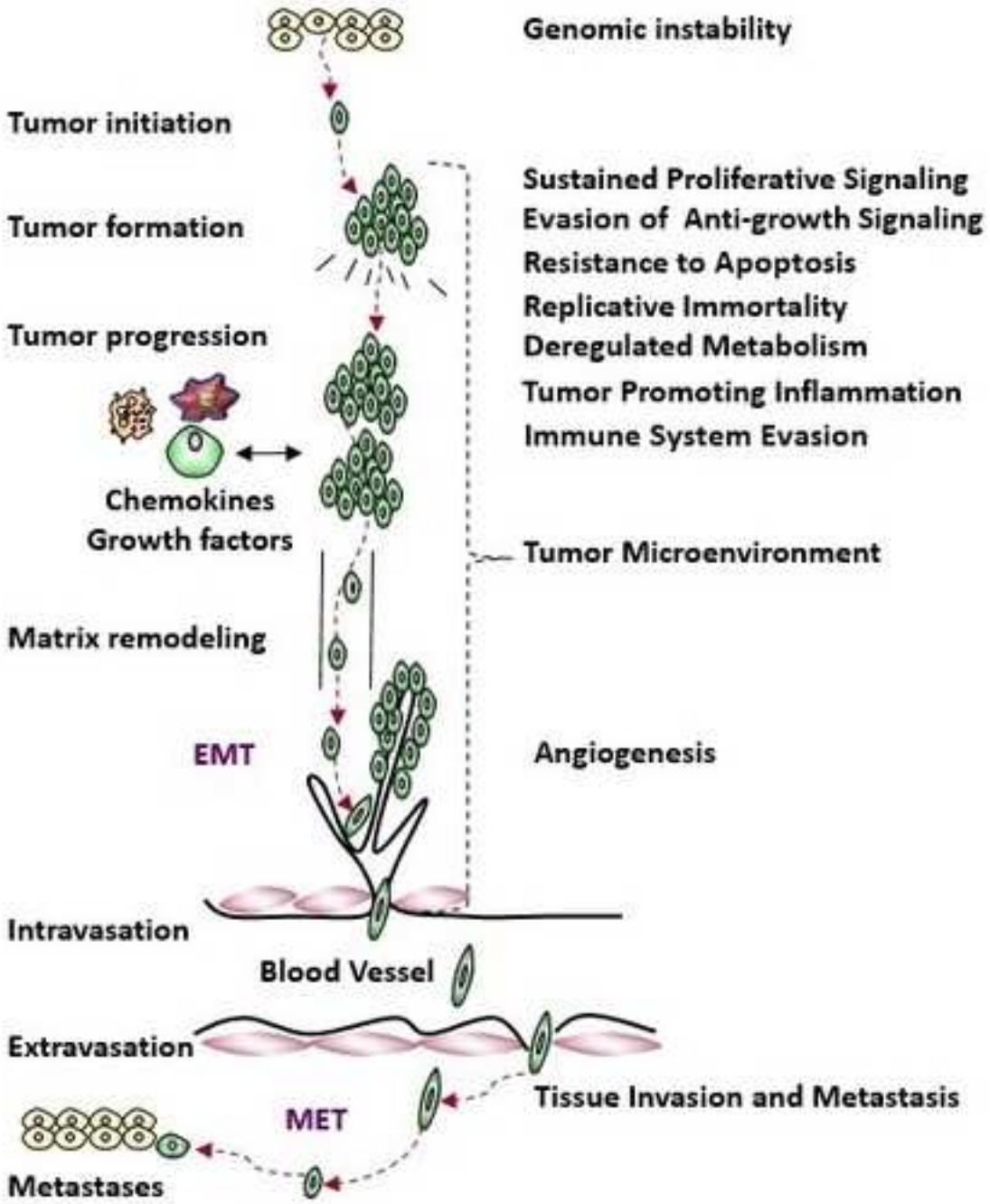

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Article Title: A Broad-Spectrum Integrative Design for Cancer Prevention and Therapy	**Author name:** R. Brooks Robey

Declarations

Seminars in Cancer Biology requires that all authors sign a declaration of conflicting interests. If you have nothing to declare in any of these categories then this should be stated.

Conflict of Interest

A conflicting interest exists when professional judgement concerning a primary interest (such as patient's welfare or the validity of research) may be influenced by a secondary interest (such as financial gain or personal rivalry). It may arise for the authors when they have financial interest that may influence their interpretation of their results or those of others. Examples of potential conflicts of interest include employment, consultancies, stock ownership, honoraria, paid expert testimony, patent applications/registrations, and grants or other funding.

Please state any competing interests

None

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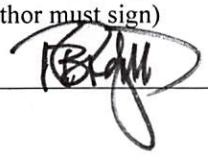

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Conflict of Interest Statement

Keith Block is an owner of the Block Center for Integrative Cancer Treatment and of North Shore Nutraceuticals; Charlotte Gyllenhaal is an employee of the Block Center for Integrative Cancer Treatment; Jack Arbiser is the inventor of US Patents involving derivatives of honokiol and NADPH oxidase inhibitors. He has also cofounded ABBY Therapeutics for the development of NADPH oxidase inhibitors; Penny Block is the Executive Director of the Block Center for Integrative Cancer Treatment and President of North Shore Nutraceuticals; Ralph J. DeBerardinis is a member of the scientific advisory boards for Peloton Therapeutics and Agios Pharmaceuticals; Anna Mae E. Diehl has grants from Shire-Research, Metabolon, and Gilead. She is also a consultant for Astrazeneca, Genentech, Japan Tobacco, and the NuSI Foundation; Byoung S. Kwon holds patents for methods regarding anti-CD 137 and adaptive CTL therapeutics; Valter D. Longo has an equity interest in L-Nutra, a company that develops medical food; Kapil Mehta is a scientific advisor to Lifecare Innovations, and holds India Patent 8.765.797, TG2 inhibitors and uses thereof; Michael P. Murphy holds intellectual property in mitochondrial therapies and has ownership shares in a company called Antipodean Pharmaceuticals Inc. which is trying to commercialize some of these compounds; Jeffrey C. Rathmell received indirect compensation from Novartis while working on this project; Luigi Ricciardiello received an unrestricted research grant from SLA Pharma AG, Switzerland.; John Stagg has a sponsored research agreement with Medimmune LLC; Matthew G. Vander Heiden is a consultant, scientific advisory board member, and owns equity in Agios Pharmaceuticals.