Ms. Rae Tian  
Assistant Editor  
CANCERS  
Re: cancers-643146

Dear Ms. Tian,

Thank you for contacting us regarding the status of our review “Combing the Cancer Genome for Novel Kinase Drivers and New Therapeutic Targets”. We have revised our previous version and addressed the concerns raised by the reviewer’s in the enclosed rebuttal letter. No major modifications have been included. Changes in the text have been tracked in red. We hope our review is now suitable for publication in Cancers.

Sincerely yours,

POINT-BY-POINT RESPONSE TO REVIEWERS COMMENTS

Reviewer 1
The authors highlighted a historical perspective of kinases including fusion gene products in carcinogenesis and cancer genomic studies that allow patients gaining access to effective treatments. Also, they draw attention to bioinformatic analysis from cancer sequencing in order to identify new oncogenic kinase drivers, and to challenges for overcoming resistance to the initial treatment.

This is a well-written review focusing on oncogenic protein kinases. A few minor revisions are listed below.

We appreciate the Reviewer’s remark that this is “a well-written review”.

1. It is not clear for me that the authors mentioned in the abstract as follows, “we highlight biomarker-based precision medicine intervention strategies that match kinase inhibitors alone or in combination to mutationally activated kinase drivers”. The reviewer would suggest such terms as “biomarker-based precision medicine” should be more clearly defined, or reworded.

We acknowledge the Reviewer’s comment and have indicated in the abstract we refer to genomic biomarker-based precision medicine strategies.

2. The details of resistance to chemotherapy are major research challenges. A couple of paper demonstrated the early tumors accumulate sub-clonal driver mutations, such as in Kras, causing intratumor heterogeneity. For the benefit of the reader, this point needs included by adding certain statements.

We agree with the Reviewer that our previous version provided limited information of the contribution of tumor heterogeneity to therapeutic resistance. We have included a paragraph, as shown below, in section 4 (page 9, lines 361-381; new references 83-86) reflecting this issue.
Lastly, intratumor heterogeneity contributes to therapy failure. It is well acknowledged that tumors are composed of heterogenous cellular populations that share a few dominant oncogenic drivers, usually acquired at the early stages of tumor development and referred to as truncal mutations. Additional mutations that are acquired in sub-clonal populations, referred to as the branch mutations, provide additional competitive advantages, such as enhanced proliferation. Tumor sub-clonality is wide-spread and found in tumors driven by potent oncogenes, including EGFR or mutant KRAS (recently revised by McGranahan and Swanton [83]). Moreover, it is hard to fully determine the exact extent of intratumor heterogeneity due to sampling bias and the genetic differences that occur as primary tumors evolve, metastasize, and establish new tumors at metastatic sties [84]. In addition to genetic heterogeneity, phenotypic heterogeneity is also a hallmark of cancer. For example, using a combinatorial siRNA screen, Yuan and colleagues found that, depending on the tissue of origin, mutant KRAS engages different effector pathways that affect their metabolic status and differentiation. As a result, tumors with the same mutation can have activation of different downstream effectors and this will impact response to treatments [85]. Importantly, anti-cancer treatments might impact the sub-clonal composition of tumors, with some subpopulations already harboring mutations that render them resistant to the anti-cancer treatment. These cells with the resistant mutation will have a competitive advantage and become the dominant clonal population in the therapy-resistant tumor. While these populations might be difficult to identify, implementation of new techniques such as serial profiling of circulating tumor cells or DNA will aid in identifying resistant sub-clonal cancer cells, and will aid in designing additional therapeutic intervention strategies aimed at targeting these cancer cells harboring drug-resistant mutations [84,86].”

3. The reviewer would suggest some of the wording should be edited. For example, on lines 34 and 69, there are repeated words in one sentence. Some typo errors should be corrected. Line 48: PymT not PyMT, which one is correct?

We thank the Reviewer for pointing out some typos or repeating words; these have been corrected or substituted by a synonym.

4. Finally, the reviewer is wondering if it can say: the authors stress “novel kinase drivers and new therapeutic targets” for cancer as mentioned in the title, but there seems to be little contents to support this. They covered oncogenic kinases and the signaling pathways which were already known to readers. The title should be more clearly expressed in line with the contents.

We thank the Reviewer’s comment; after addressing all Reviewer’s concerns we decided to keep the title.

Reviewer 2
The review entitled "Combing the Cancer Genome for Novel Kinase Drivers and New Therapeutic Targets" provides an overview of protein kinases as therapeutic targets in cancer highlighting the genetic mechanisms leading to increased protein kinase activity, the progress for clinical developments of kinase-targeted strategies and the challenges for overcoming tumor resistance to small molecule kinase inhibitors. This is a nice paper overall, well written and informative. Figures are accurate. It has a good and balanced choice of recent reviewed papers.

We appreciate the Reviewer considers this is a well written and informative review and that figures are accurate.

I have only few minor suggestions:
1. Other gene fusions resulting in constitutive kinase activation, in addition to BCR-ABL and EML4-ALK, and corresponding inhibitors should be reported or at least the authors should refer to recent reviews reporting additional kinase fusions.

We thank the Reviewer’s suggestion and have included information of additional kinase fusions including ROS1 and RET (page 3, lines 118-125), as well as additional references (new references 21, 24 and 25). It now reads:

“Additional gene fusions that result in constitutive kinase activation have been detected in other cancer types [21]. For example, the EML4-ALK gene fusion is present in 3-5% of non-small cell lung cancer (NSCLC) cases [22]. In clinical trials, the ALK inhibitor crizotinib showed greater benefit than chemotherapy in NSCLC patients presenting with ALK gene rearrangement, resulting in the approval of crizotinib for the treatment of EML4-ALK positive NSCLC patients (approximately 70,000 patients diagnosed annually worldwide) [23]. ROS1 gene fusions are found in 1-2% of NSCLC cases as well as in cholangiocarcinoma, glioblastoma, or colorectal cancer, and can be targeted with crizotinib [24]. In addition, RET gene fusions have been identified in NSCLC and thyroid carcinoma [25].”

2. Lines 195 to 199, The authors should reformulate this sentence to avoid misunderstanding, since Avastin is not directed against overexpressed RTKs but it functions by ligand trapping.

We appreciate the Reviewer’s remark and have re-written the corresponding sentence to clarify that Avastin binds to the receptor ligand (page 5, lines 205-211):

“After this discovery, additional monoclonal antibodies directed against overexpressed receptor tyrosine kinases or their ligands have been approved for the treatment of cancer, including cetuximab, approved for the treatment of head and neck squamous cell carcinoma (HNSCC) targeting EGFR and bevacizumab (Avastin), which targets the vascular endothelial growth factor A (VEGF-A), a ligand of the VEGF receptor. Importantly, these are examples of the earliest immunotherapeutic treatments against cancer as these agents can activate the immune system, which can promote tumor cell clearance [40].”

3. In the paragraph 2.3, the Authors should also add AXL (Martinelli E et al Oncotarget 2015; Ohshima K et al Sci. Rep. 2017) as additional amplified RTK in cancer. Kinase domain duplication is another oncogenic driver in cancer that should be mentioned (i.e. Gallant JN et al Cancer Discov 2015).

As per the Reviewer’s suggestion, we have included AXL amplification (lines 198-202) as well as examples of kinase domain duplications as oncogenic drivers (lines 219-221).

As stated on page 5 lines 198-202:

“Additional amplified RTKs in cancer include EGFR (7p11.2), FGFR1 (Fibroblast Growth Factor Receptor 1; 8p11.23), PDGFRα (Platelet-derived Growth Factor Receptor alpha; 4q12), MET (a.k.a. HGFR, Hepatocyte Growth Factor Receptor; 7q31.2), FLT3 (FMS Related Tyrosine kinase 3; 13q12.2) and AXL (Tyrosine-protein kinase receptor UFO; 19q13.2) [28,39].”

As stated on page 5, lines 219-221:
Lastly, tandem kinase domain duplications in kinases such as EGFR, BRAF and FGFR1 are rare alterations (<1%) that also promote tumorigenesis [45-47].

**Reviewer 3**

The authors present a relevant and timely review of the identification, functional characterization, and resistance mechanisms associated with oncogenic kinase targets. The inclusion of historical context and specific kinase examples are useful and proportionally appropriate for each section. Below are minor comments for the authors to consider.

We thank the Reviewer found our review relevant and timely.

1. The specific examples given in each section encompass only solid tumors. To more broadly represent progress in this field, examples could also be included from leukemias/lymphomas as there are many such oncogenic kinase genetic events and resistance to kinase inhibitors that could be mentioned.

We agree with the Reviewer that our previous version mainly included examples from solid tumors. We have now included examples covering hematological malignancies (Page 4, lines 152-156; page 6, lines 244-253. New references 32, 52 and 57).

As state on page 4, lines 152-156:

> “Gain-of-function mutations in protein kinases genes are also frequently found in hematologic malignancies. For example, 30% of patients with acute myeloid leukemia (AML) harbor activating mutations in the FLT3 receptor tyrosine kinase (RTK). Midostaurin, a multi-targeted kinase inhibitor that targets FLT3, gained FDA-approval for AML patients with FLT3 mutations in 2017 [32].”

As state on page 6, lines 244-253:

> “siRNA- and CRISPR-based genome-wide screenings have been seminal in the identification of genetic drivers in both solid tumors and hematologic malignancies [52] [53]. These approaches allow for identification of non-mutated actionable vulnerabilities. For example, CDK9 has been identified as a dependency in hepatocellular carcinoma that cooperates with Myc to sustain cancer cell survival [54]. Similarly, Wee1 is a synthetic lethal dependency in p53-mutant head and neck squamous cell carcinoma [55], and several non-mutated tyrosine kinases, such as CSF-1R or ROR1, have been identified as dependencies in leukemia patients [52]. Moreover, genome-wide screenings are being used in in vivo and preclinical cancer models that will lead to discovery of novel actionable alterations or more efficacious combination therapies [56,57].”

2. The issue of tumor heterogeneity is briefly mentioned in concluding remarks on line 445, but should be also be specifically mentioned in Section 4 for its proposed role in resistance to therapy.

We appreciate the Reviewer’s suggestion and have expanded on the contribution of tumor heterogeneity to therapeutic resistance (page 9, lines 361-381; new references 83-86).

> “Lastly, intratumor heterogeneity contributes to therapy failure. It is well acknowledged that tumors are composed of heterogenous cellular populations that share a few dominant oncogenic drivers, usually acquired at the early stages of tumor development and referred to as truncal mutations. Additional mutations that are acquired in sub-clonal populations, referred to as the branch mutations, provide
additional competitive advantages, such as enhanced proliferation. Tumor sub-clonality is wide-spread and found in tumors driven by potent oncogenes, including EGFR or mutant KRAS (recently revised by McGranahan and Swanton [83]). Moreover, it is hard to fully determine the exact extent of intratumor heterogeneity due to sampling bias and the genetic differences that occur as primary tumors evolve, metastasize, and establish new tumors at metastatic sites [84]. In addition to genetic heterogeneity, phenotypic heterogeneity is also a hallmark of cancer. For example, using a combinatorial siRNA screen, Yuan and colleagues found that, depending on the tissue of origin, mutant KRAS engages different effector pathways that affect their metabolic status and differentiation. As a result, tumors with the same mutation can have activation of different downstream effectors and this will impact response to treatments [85]. Importantly, anti-cancer treatments might impact the sub-clonal composition of tumors, with some subpopulations already harboring mutations that render them resistant to the anti-cancer treatment. These cells with the resistant mutation will have a competitive advantage and become the dominant clonal population in the therapy-resistant tumor. While these populations might be difficult to identify, implementation of new techniques such as serial profiling of circulating tumor cells or DNA will aid in identifying resistant sub-clonal cancer cells, and will aid in designing additional therapeutic intervention strategies aimed at targeting these cancer cells harboring drug-resistant mutations [84,86].”

3. Section 3, lines 252-255 – The authors list implementing gene expression profiling and signaling pathway analysis as challenges. However, I am curious whether the authors see a role for transcriptional or proteomic analyses in identifying kinase pathway targets in the clinical setting? Section 3 – it may be necessary to caution that DNA-only genomic screening may identify genes that are mutated but not expressed in a particular tumor type.

We thank the Reviewer’s comment and have highlighted that “sole DNA sequencing identifies mutated genes but these might not be expressed in the corresponding tumor” (page 6, lines 274-278). Previous work from our lab showed that DNA sequencing can be complemented by proteomic analysis (i.e. RPPA) to identify actionable alterations in lack of detection of mutated drivers. We consider that RNAseq and proteomic analysis (i.e. mass spectrometry and RPPA) will complement next-generation sequencing to evaluate expression of potential drivers or activation of oncogenic signaling networks that will allow identification of additional therapeutic targets.

As state on page 6, lines 274-278:

“DNA sequencing alone only identifies mutated genes, but these might not be expressed in the tumor cells and may represent passenger mutations. Optimization of technologies such as RNAseq or proteomic analysis, including mass spec, may be required in the clinical setting and will aid in the identification of novel actionable vulnerabilities [59-61].”

4. Lines 352-355 – should mention the limitations in xenograft and organoid models for identifying toxicities and interactions with the host immune system when screening combinatorial and novel therapies.

As per the Reviewer’s suggestion, we have included an statement on the limitations of patient-derived models and how this might be overcome (page 9, lines 400-403; new references 92 and 93). It reads:

“While initially these models did not allow for testing of immunotherapies, the establishment of humanized PDX models with a reconstituted immune system [92] or organoids that retain cells from the tumor microenvironment [93] might help overcome some limitations of these models.”
Reviewer 4

This manuscript focuses on protein kinases and their role/deregulation in cancer. It is very well written review that provides both basic information and examples of precision therapies in cancer. Overall, the manuscript would be of interest to the readership of CANCERS. However, I think that the title is a little misleading. The authors only briefly mention about approaches for defining new kinase drivers (section 3). This part should be expanded. Several recent screenings have identified many new genetic drivers in solid tumors, as well as in lymphoma.

We appreciate the Reviewer found our review was very well-written and found it of the interest of the reader of CANCERS. Following the Reviewer’s comment, we have expanded Section 3 to include details of loss-of-function screenings and have referenced examples covering solid tumors and hematological malignancies (page 4, lines 152-156; page 6, lines 244-253. New references 32, 52-57).

As state on page 4, lines 152-156:

“Gain-of-function mutations in protein kinases genes are also frequently found in hematologic malignancies. For example, 30% of patients with acute myeloid leukemia (AML) harbor activating mutations in the FLT3 receptor tyrosine kinase (RTK). Midostaurin, a multi-targeted kinase inhibitor that targets FLT3, gained FDA-approval for AML patients with FLT3 mutations in 2017 [32].”

As state on page 6, lines 244-253:

“siRNA- and CRISPR-based genome-wide screenings have been seminal in the identification of genetic drivers in both solid tumors and hematologic malignancies [52] [53]. These approaches allow for identification of non-mutated actionable vulnerabilities. For example, CDK9 has been identified as a dependency in hepatocellular carcinoma that cooperates with Myc to sustain cancer cell survival [54]. Similarly, Wee1 is a synthetic lethal dependency in p53-mutant head and neck squamous cell carcinoma [55], and several non-mutated tyrosine kinases, such as CSF-1R or ROR1, have been identified as dependencies in leukemia patients [52]. Moreover, genome-wide screenings are being used in in vivo and preclinical cancer models that will lead to discovery of novel actionable alterations or more efficacious combination therapies [56,57].”

Additional comments:
1. Figure 3 is difficult to read – text/labels are barely visible.

We thank the Reviewer noticed that figure labels were difficult to read and have increased the font size in the revised version.