EMBO
Molecular Medicine

Fatty Acid Synthase mediates EGFR palmitoylation in EGFR mutated Non Small Cell Lung Cancer

Azhar Ali, Elena Levantini, Teo Jun Ting, Julian Goggi, John G. Clohessy, Wu Chan Shuo, Polly Chen Leilei, Henry Yang, Indira Krishnan, Olivier Kocher, Zhang Junyan, Ross Soo, Kishore Bhakoo, Chin Tan Min, Daniel G. Tenen

Transaction Report:

(Note: With the exception of the correction of typographical or spelling errors that could be a source of ambiguity, letters and reports are not edited. The original formatting of letters and referee reports may not be reflected in this compilation.)

1st Editorial Decision   06 September 2017

Thank you for the submission of your manuscript to EMBO Molecular Medicine.

I sincerely apologise for the very unusual delay in getting back to you on your manuscript. In fact, we experienced significant difficulties in securing expert and willing reviewers, and then obtaining their evaluations in a timely fashion, mostly due to the overlapping holiday season. Furthermore, I failed to retrieve a third evaluation for this manuscript. To avoid further unproductive delays, I am making a decision based on the two available evaluations.

As you will see, while reviewer 2 provides a thoughtful and detailed critique, the evaluation by #reviewer 1 is positive but not very informative. After internal discussion, it was agreed that reviewer 2 raises many important points that would need to be addressed including 1) the concern that the observed effects of Gefitinib might be due to off-target activity, 2) the need for further evidence to demonstrate that FASN up-regulation in an EGFR resistance cell line is a causal effect, 3) the lack of important controls. Reviewer 2 also notes the need for further experimentation to demonstrate the translational potential of Orlistat re-purposing for NSCLC. We agree that the latter issue needs to be clarified to bring the manuscript to the level expected for an EMBO Molecular medicine article. I wish to stress our impression that notwithstanding the significant elements of concern, reviewer 2 is in essence quite appreciative of the study.

In conclusion, while publication of the paper cannot be considered at this stage, we are willing to consider a substantially revised manuscript, addressing the reviewer's concerns including with further experimentation where required.

***** Reviewer's comments *****

Referee #1 (Remarks for Author):

Ali et al have investigated the TKI-resistance in mutant EGFR NSCLC demonstratING that this is mediated by FASn palmitoylation. Interestingly this reaction can be efficiently targeted in cell culture and in xenografts.

This is an important topic in view of the few treatments available for NSCLC patients. The experiments are well designed, the results clearly written and the figures well understandable.

The AA provide evidence for a functional interrelationship between mutated EGFR and FASN and show that the fatty acid pathway is a candidate target for acquired TKI resistance in EGFR mutant NSCLC patients. This is an important finding that deserves public knowledge at a high level. Moreover, they prove that since the TKI resistant tumor cells rely on EGFR palmitoylation, they can be targeted by Orlistat, an FDA approved anti-obesity drug.

Referee #2 (Remarks for Author):

The authors study the role of palmitoylation in the development of resistance to EGFR TKI inhibitor targeted therapy and the regulation of this palmitoylation by SRBP1 and FASN through EGFR signaling resistance mechanism. They find that, in lung cancer, mutated EGFR mediates TKI resistance through regulation of FASN. The authors also report that TKI resistance can be circumvented by treatment with the FASNi orlistat in cell lines and in transgenic mouse models. The topic is of great interest for several reasons including a totally new pathway to EGFR TKI inhibitor resistance, and also a unique dependency on SRBP1 and FASN that appears to be specific for EGFR mutated lung cancers.

Comments to the Authors;
The paper is reviewed in the context of the potential importance for clinical translation and for elucidation of a new fatty acid synthesis pathway involved in EGFR targeted therapy. The experiments are technically well done and clearly presented. However, there are several issues the authors need to deal with.
1. Several findings are not novel and the primary papers are not discussed. As the author note, it has already been reported that wild type EGFR is palmitoylated. However, the functional role of EGFR palmitoylation is controversial. For instance, Runkle et al, Mol Cell (2016) showed that palmitoylation negatively regulates EGFR; palmitoylated EGFR is ubiquitinated and fails to traffic to endosomes. By contrast, Bollu et al. Oncotarget (2015), reported that EGFR is activated by FASN-dependent palmitoylation. To clarify these controversial findings, it would be useful if the authors discussed their results in the context of previous literature.
2. The preponderance of the data provided by the authors indicate that EGFR signaling activates fatty acid synthesis (as already documented in the literature), however the authors have not provided sufficient evidence to support their assertion that palmitoylation is required specifically for the signaling of TKI resistant EGFR.
3. Their conclusions are almost entirely based on the study of one EGFR mutant TKI resistant NSCLC line. Because of the importance for clinical translation it is very important to determine if their therapeutic results with orlistat are evident in studies of other NSCLC lines including EGFR mutant, EGFR mutant TKI resistant, and EGFR wildtype NSCLCs. This is particularly true for the xenograft studies. Orlistat may be a potential drug that can be "repurposed" for NSCLC treatment, but the mechanisms may go beyond those focused on in this report and thus could work in a variety of NSCLCs, and/or not work in some EGFR mutant NSCLCs. While they do not have to provide tests on large numbers of xenografts at least some others should be included. Following along this line of thought, for clinical translation, the question naturally arises what if orlistat was added in during the primary xenograft treatment of EGFR mutant NSCLC? Would this prevent the development of resistance? Such data, whichever way it came out, would greatly add to the clinically translational value of this paper.
3. The authors also do not provide sufficient evidence that orlistat reaches its target in their in vivo experiments (see below).

There are also a series of specific points that authors should also deal with that are summarized (not in order of priority):

1. Line 122. The authors should provide the primary data that "DNA from PC-9GR cells was negative from T790M mutations or MET amplification" or cite the appropriate reference;

2. Line 133. The observation that FASN is upregulated in a single EGFR resistant cell line may be a fortuitous event. It would be of interest to know the sensitivity of a larger panel of NSCLC cells lines to FASNi or silencing.

3. Line 140: PC-9 should be named PC-9GR

4. Line 151: given the importance of the knockdown experiments, the phenotype of the RNAi experiment should be "rescued" with an RNAi resistant cDNA (to rule out off-target effects)

5. Line 161: the authors show that getifinib, but not EGFR silencing, increases FFA in EGFR resistant cells. One interpretation could be that this reflects a getifinib off target effect rather that by EGFR inhibition. The authors need to discuss. In the same manner it would be useful to show with another EGFR TKI, erlotinib, that their findings hold.

6. Given that all of the experiments relied on on EGFR mutant NSCLC derivative, PC-9Gr, it is possible the effects described could be an idiosyncratic phenomenon that occurs only in PC-9GR. Thus, it would be appropriate for the authors to test other NSCLC EGFR mutant cell lines that acquired getifinib resistance. In addition, in reviewing the Methods section, there is no statement that the cell lines were DNA fingerprinted or mycoplasma tested. While I think it is highly unlikely that such events occurred, they need to be definitely accounted for. In fact, one event that could explain a lot of their findings, would be mycoplasma contamination of some of the EGFR resistant derivatives.

7. Line 174: The sentence: overall these data show a positive correlation between getifinib resistance and elevated SREBP1 and FASN" is misleading. A better wording of the sentence would state:" the data indicate that EGFR positively regulates...". This line is also redundant because this concept is also restated at lane 181.

8. Line 200 (fig. 2C): again getifinib increases FFA, but RNAi EGFR knockdown does not cause the same effect. This finding raises the question that FFA elevation is a getifinib off target effect

9. Line 212. The fact that wild type EGFR does not overtly upregulates SREBP and FASN could be due to the fact that wild type EGFR is not constitutively activated. What is the effect if wild-type EGFR is stimulated with EGF?

10. Line 254; could it be that the lack of growth promoting effects of wild type EGFR is merely due to the fact that it is not constitutively active? In other words, couldn't it be that wild type EGFR stimulates FASN, but at a lower level than constitutively active EGFR? Again EGF treatment may clarify this point.

11. Line 280. EGFR Palmitoylation was already reported to occur. The authors should discuss their findings in relation to the literature.

12. Line 302. The conclusion is based on correlative data. While this would require additional experimentation, structural studies or studies with recombinant proteins in cell free conditions are needed to prove the assertion that mutant EGFR is preferentially palmitoylated.

13. Line 313. The authors should discuss their findings in the context of the literature.

14. Line 365: The conclusion regarding the dependency on FASN is based on the effect of orlistat in NL20 cells, a correlative finding. What happens if NL20 cells are treated with EGF?

15. Line 377 and 395. An important experiment to try (no matter what the result) would be to try to rescue he phenotype of orlistat and/or FASN silencing by administration of exogenous palmitate.

16. Line 408: Given the clinical translational importance of this key experiment, it would be of great value to the paper if the authors could demonstrate that orlistat inhibits FASN in vivo either by performing enzymatic assays or by measuring directly fatty acids in tumors.

17. Line 408 and 452. In my opinion the data presented are insufficient to establish circulating FASN as a biomarker (for instance where is FASN coming from? Exosomes? Why circulating FASN is down-regulated by orlistat treatment?). In my opinion this part is not necessary in the paper.

1st Revision - authors' response   **12 November 2017**

Referee #1 (Remarks for Author):

Ali et al have investigated the TKI-resistance in mutant EGFR NSCLC demonstrating that this is mediated by FASn palmitoylation. Interestingly this reaction can be efficiently targeted in cell culture and in xenografts.

This is an important topic in view of the few treatments available for NSCLC patients. The experiments are well designed, the results clearly written and the figures well understandable.

The AA provide evidence for a functional interrelationship between mutated EGFR and FASN and show that the fatty acid pathway is a candidate target for acquired TKI resistance in EGFR mutant NSCLC patients. This is an important finding that deserves public knowledge at a high level. Moreover, they prove that since the TKI resistant tumor cells rely on EGFR palmitoylation, they can be targeted by Orlistat, an FDA approved anti-obesity drug.

Referee #2 (Remarks for Author):

The authors study the role of palmitoylation in the development of resistance to EGFR TKI inhibitor targeted therapy and the regulation of this palmitoylation by SRBP1 and FASN through EGFR signaling resistance mechanism. They find that, in lung cancer, mutated EGFR mediates TKI resistance through regulation of FASN. The authors also report that TKI resistance can be circumvented by treatment with the FASNi orlistat in cell lines and in transgenic mouse models. The topic is of great interest for several reasons including a totally new pathway to EGFR TKI inhibitor resistance, and also a unique dependency on SRBP1 and FASN that appears to be specific for EGFR mutated lung cancers.

We would like to thank both reviewers for their comments and recommendations.

Comments to the Authors;
The paper is reviewed in the context of the potential importance for clinical translation and for elucidation of a new fatty acid synthesis pathway involved in EGFR targeted therapy. The experiments are technically well done and clearly presented. However, there are several issues the authors need to deal with.

1. Several findings are not novel and the primary papers are not discussed. As the author note, it has already been reported that wild type EGFR is palmitoylated. However, the functional role of EGFR palmitoylation is controversial. For instance, Runkle et al, Mol Cell (2016) showed that palmitoylation negatively regulates EGFR; palmitoylated EGFR is ubiquitinated and fails to traffic to endosomes. By contrast, Bollu et al. Oncotarget (2015), reported that EGFR is activated by FASN-dependent palmitoylation. To clarify these controversial findings, it would be useful if the authors discussed their results in the context of previous literature.

*We have inserted the requested information in the current version of the manuscript, from **lines 582 to 596** of the discussion, hypothesizing reasons for the differences observed between our study and that of others on the effects of EGFR palmitoylation.*

2. The preponderance of the data provided by the authors indicate that EGFR signaling activates fatty acid synthesis (as already documented in the literature), however the authors have not provided sufficient evidence to support their assertion that palmitoylation is required specifically for the signaling of TKI resistant EGFR.

*Following the Reviewer's suggestion, we performed cell rescue assays, with the supplementation of palmitate after 2BP treatment in PC-9P, PC-9GR, NL20, and H1975 cells to support the evidence that palmitoylation is important for survival of TKI resistant cells (please see **lines 359 to 365; and Fig 4E**).*

3. Their conclusions are almost entirely based on the study of one EGFR mutant TKI resistant NSCLC line. Because of the importance for clinical translation it is very important to determine if their therapeutic results with orlistat are evident in studies of other NSCLC lines including EGFR mutant, EGFR mutant TKI resistant, and EGFR wildtype NSCLCs. This is particularly true for the xenograft studies. Orlistat may be a potential drug that can be "repurposed" for NSCLC treatment, but the mechanisms may go beyond those focused on in this report and thus could work in a variety of NSCLCs, and/or not work in some EGFR mutant NSCLCs. While they do not have to provide tests on large numbers of xenografts at least some others should be included. Following along this

line of thought, for clinical translation, the question naturally arises what if orlistat was added in during the primary xenograft treatment of EGFR mutant NSCLC? Would this prevent the development of resistance? Such data, whichever way it came out, would greatly add to the clinically translational value of this paper.

*We have included data obtained from another EGFR mutated NSCLC cell line with acquired TKI resistance, HCC4006GR (EGFRdel746-749/A750P), in which EGFR-FASN signaling is active, whereas the parental line HCC4006P has no such active molecular pathway (**Lines 180 to 195; and Fig S6**). We treated HCC4006GR, together with the TKI resistant EGFR mutated cell lines H1650 (EGFR del746-750) and H820 (EGFRdel746-750/T790M), with Orlistat to demonstrate its efficacy in TKI resistant EGFR mutated NSCLC (**Fig S2**). With regards to the question if Orlistat can delay Gefitinib resistance, we thank the Reviewer for this important question that we will address as part of a follow-up study, as it requires a considerable amount of time to obtain this data.*

3. The authors also do not provide sufficient evidence that orlistat reaches its target in their in vivo experiments (see below).

*We carried out FFA measurement on xenograft tumors (**Fig 6B**) and transgenic models (**Fig 7C**), and examined FASN protein expression in transgenic tumors (**Fig 7D**), demonstrating that Orlistat reaches tumors and affects cellular fatty acid and FASN levels.*

There are also a series of specific points that authors should also deal with that are summarized (not in order of priority):

1. Line 122. The authors should provide the primary data that "DNA from PC-9GR cells was negative from T790M mutations or MET amplification" or cite the appropriate reference;

*We carried out DNA analyses on EGFR T790M mutation and MET amplification on PC-9GR and HCC4006GR cells and we included these data, as requested in **Appendix Fig S1**.*

2. Line 133. The observation that FASN is upregulated in a single EGFR resistant cell line may be a fortuitous event. It would be of interest to know the sensitivity of a larger panel of NSCLC cells lines to FASNi or silencing.

*We tested the sensitivity of TKI resistant EGFR mutated HCC4006GR, H1650, and H820 cells to Orlistat and demonstrated that FASN plays a role in the survival of these resistant cells (**Lines 109 to 115; Fig S2**).*

3. Line 140: PC-9 should be named PC-9GR

We apologize for not making it clear, and that PC-9 is the correct label as isogenic PC-9 cells refers to both parental and resistant cells.

4. Line 151: given the importance of the knockdown experiments, the phenotype of the RNAi experiment should be "rescued" with an RNAi resistant cDNA (to rule out off-target effects)

*To address this, we created EGFRi#1, a RNAi resistant EGFR cDNA clone to determine the specificity of EGFRi#1 siRNAs, demonstrating that the siRNAs are specific against their intended target (**Lines 131 to139; Fig S3C**).*

5. Line 161: the authors show that gefitinib, but not EGFR silencing, increases FFA in EGFR resistant cells. One interpretation could be that this reflects a getifinib off target effect rather that by EGFR inhibition. The authors need to discuss. In the same manner it would be useful to show with another EGFR TKI, erlotinib, that their findings hold.

*To address this issue, we examined the sensitivity of PC-9P, PC-9GR, HCC4006P, and HCC4006GR cells to Erlotinib, and compared cellular FFAs levels between vehicle- and Erlotinib-treated cells. Our data show that the elevated FFAs seen is not a consequence of Gefitinib off-target effects but an observation seen in the resistant cells against TKI inhibitors (**Lines 157 to 161; Fig S5A and S5B; and Lines 191 to 195; Fig S7A and S7B**).*

6. Given that all of the experiments relied on EGFR mutant NSCLC derivative, PC-9Gr, it is possible the effects described could be an idiosyncratic phenomenon that occurs only in PC-9GR. Thus, it would be appropriate for the authors to test other NSCLC EGFR mutant cell lines that acquired getifinib resistance. In addition, in reviewing the Methods section, there is no statement that the cell lines were DNA fingerprinted or mycoplasma tested. While I think it is highly unlikely that such events occurred, they need to be definitely accounted for. In fact, one event that could explain a lot of their findings, would be mycoplasma contamination of some of the EGFR resistant derivatives.

*To address Reviewer's comment, TKI resistant EGFR mutated HCC4006GR, H820, and H1650 cells were included in this study. Acquired resistant HCC4006GR exhibited the EGFR-FASN signaling axis, similar to that in PC-9GR cells. All three cell lines were found resistant to both gefitinib and erlotinib, whereas their growth could be suppressed by FASN inhibition. We tested all cell lines used in this study for mycoplasma (**Fig S18**) and DNA fingerprinted (**Supplementary File 2**).*

7. Line 174: The sentence: overall these data show a positive correlation between getifinib resistance and elevated SREBP1 and FASN" is misleading. A better wording of the sentence would state:" the data indicate that EGFR positively regulates...". This line is also redundant because this concept is also restated at lane 181.

*We have amended the text, as advised (**Lines 173 to 174**) by rephrasing it into – ".....these data indicate that EGFR positively regulates SREBP1 and FASN expression in acquired TKI resistant EGFR mutated NSCLC....."*

8. Line 200 (fig. 2C): again getifinib increases FFA, but RNAi EGFR knockdown does not cause the same effect. This finding raises the question that FFA elevation is a getifinib off target effect.

*We tested H1975 and H1703 cells with Erlotinib, another TKI inhibitor, and compared FFAs between vehicle- and Erlotinib-treated cells and demonstrated that the elevated FFAs is not likely due to gefitinib off-target effects (**Lines 217 to 219, Fig 7D**).*

9. Line 212. The fact that wild type EGFR does not overtly upregulates SREBP and FASN could be due to the fact that wild type EGFR is not constitutively activated. What is the effect if wild-type EGFR is stimulated with EGF?

*We investigated the effects of EGFR activation by EGF on SREBP1 and FASN expression in H1703 cells (**Lines 231 to 238; Fig S8**) and demonstrated that EGFR mutant proteins, and not wild-type, regulate SREBP1 and FASN. We thank the Reviewer for this observation that allowed us to strengthen our observation through these carefully designed controls.*

10. Line 254; could it be that the lack of growth promoting effects of wild type EGFR is merely due to the fact that it is not constitutively active? In other words, couldn't it be that wild type EGFR stimulates FASN, but at a lower level than constitutively active EGFR? Again EGF treatment may clarify this point.

Please find our response in our point #9 where we demonstrated that EGFR mutant proteins, and not wild-type, regulate SREBP1 and FASN expression.

11. Line 280. EGFR Palmitoylation was already reported to occur. The authors should discuss their findings in relation to the literature.

*We did acknowledge reports regarding EGFR palmitoylation (**lines 308-310 and 582 to 596**). Please also see our response to author query 13.*

12. Line 302. The conclusion is based on correlative data. While this would require additional experimentation, structural studies or studies with recombinant proteins in cell free conditions are needed to prove the assertion that mutant EGFR is preferentially palmitoylated.

*We performed cell-free palmitoylation assays to determine if mutant EGFR is preferentially palmitoylated, and our data demonstrated that EGFR mutants are preferentially palmitoylated over wild-type proteins (please see **Lines 329 to 337; and Fig 4C**).*

13. Line 313. The authors should discuss their findings in the context of the literature.

*We followed the Reviewer's concern and discussed these findings in the Discussion section (**lines 582 to 596**).*

14. Line 365: The conclusion regarding the dependency on FASN is based on the effect of orlistat in NL20 cells, a correlative finding. What happens if NL20 cells are treated with EGF?

*To determine if the lack of dependency on FASN is attributed to lack of constitutive EGFR activity in wild-type NL20 cells, cells were stimulated with EGF prior Orlistat treatment, and our data indicate that stimulation of EGFR activity does not affect NL20's lack of sensitivity to Orlistat (please see **Lines 409 to 416; Fig S13A and S13B**).*

15. Line 377 and 395. An important experiment to try (no matter what the result) would be to try to rescue he phenotype of orlistat and/or FASN silencing by administration of exogenous palmitate.

*We carried out rescue assay by supplementation of palmitate to FASN knockdown PC-9GR, H1975, and H1703 cells, and our data demonstrated that exogenous palmitate improved the growth of FASNi-treated cells, thus reinforcing our initial observations (please see **Lines 428 to 432; Fig S15**).*

16. Line 408: Given the clinical translational importance of this key experiment, it would be of great value to the paper if the authors could demonstrate that orlistat inhibits FASN in vivo either by performing enzymatic assays or by measuring directly fatty acids in tumors.

*We measured tumor FFAs from xenograft (**Fig 6B**) and transgenic mice (**Fig 7C**), and examined FASN protein expression in tumors from transgenic mice (**Fig 7D**), demonstrating that Orlistat inhibits FASN and affects FFAs levels in tumors.*

17. Line 408 and 452. In my opinion the data presented are insufficient to establish circulating FASN as a biomarker (for instance where is FASN coming from? Exosomes? Why circulating FASN is down-regulated by Orlistat treatment?). In my opinion this part is not necessary in the paper.

Following the Reviewer's suggestions, we decided to remove our previous statement on circulating FASN as biomarkers.

2nd Editorial Decision	13 December 2017

Thank you for the submission of your revised manuscript to EMBO Molecular Medicine. We have now received the enclosed report from the referee who was asked to re-assess it. As you will see the reviewer is now globally supportive and I am pleased to inform you that we will be able to accept your manuscript pending a few final editorial amendments.

***** Reviewer's comments *****

Referee #1 (Comments on Novelty/Model System for Author):

This is the second review. The improvements made in the manuscript make it very novel, highly technical and high on medical impact. The system is adequate this is at a pre-clinical level.

Referee #1 (Remarks for Author):

As requested by the Editor I have scrutinised the manuscript not only following my personal

criticisms but also, and in depth, those raised by reviewer 2.

The authors have done an immense amount of experimentation answering all the points raised. I feel extremely satisfied for their answers and for their their experiments and for their modifications of the manuscript.

The only point that the AA do not address is whether "Orlistat can delay Gefitinib resistance". I agree with the AA that the amount of time required required for this experiment makes it unsuitable at the level of a manuscript review.

In addition, I believe that this is not a question that should have been asked, as the answer would belong to a totally different project.

EMBO PRESS

YOU MUST COMPLETE ALL CELLS WITH A PINK BACKGROUND ⬇

PLEASE NOTE THAT THIS CHECKLIST WILL BE PUBLISHED ALONGSIDE YOUR PAPER

Corresponding Author Name: Dr Daniel Tenen
Journal Submitted to: EMBO Molecular Medicine
Manuscript Number: EMM-2017-08313

Reporting Checklist For Life Sciences Articles (Rev. June 2017)

This checklist is used to ensure good reporting standards and to improve the reproducibility of published results. These guidelines are consistent with the Principles and Guidelines for Reporting Preclinical Research issued by the NIH in 2014. Please follow the journal's authorship guidelines in preparing your manuscript.

A- Figures
1. Data
The data shown in figures should satisfy the following conditions:

➜ the data were obtained and processed according to the field's best practice and are presented to reflect the results of the experiments in an accurate and unbiased manner.
➜ figure panels include only data points, measurements or observations that can be compared to each other in a scientifically meaningful way.
➜ graphs include clearly labeled error bars for independent experiments and sample sizes. Unless justified, error bars should not be shown for technical replicates.
➜ if n< 5, the individual data points from each experiment should be plotted and any statistical test employed should be justified
➜ Source Data should be included to report the data underlying graphs. Please follow the guidelines set out in the author ship guidelines on Data Presentation.

2. Captions

Each figure caption should contain the following information, for each panel where they are relevant:

➜ a specification of the experimental system investigated (eg cell line, species name).
➜ the assay(s) and method(s) used to carry out the reported observations and measurements
➜ an explicit mention of the biological and chemical entity(ies) that are being measured.
➜ an explicit mention of the biological and chemical entity(ies) that are altered/varied/perturbed in a controlled manner.

➜ the exact sample size (n) for each experimental group/condition, given as a number, not a range;
➜ a description of the sample collection allowing the reader to understand whether the samples represent technical or biological replicates (including how many animals, litters, cultures, etc.).
➜ a statement of how many times the experiment shown was independently replicated in the laboratory.
➜ definitions of statistical methods and measures:
  • common tests, such as t-test (please specify whether paired vs. unpaired), simple χ^2 tests, Wilcoxon and Mann-Whitney tests, can be unambiguously identified by name only, but more complex techniques should be described in the methods section;
  • are tests one-sided or two-sided?
  • are there adjustments for multiple comparisons?
  • exact statistical test results, e.g., P values = x but not P values < x;
  • definition of 'center values' as median or average;
  • definition of error bars as s.d. or s.e.m.

Any descriptions too long for the figure legend should be included in the methods section and/or with the source data.

In the pink boxes below, please ensure that the answers to the following questions are reported in the manuscript itself. Every question should be answered. If the question is not relevant to your research, please write NA (non applicable). We encourage you to include a specific subsection in the methods section for statistics, reagents, animal models and human subjects.

B- Statistics and general methods

Please fill out these boxes ⬇ (Do not worry if you cannot see all your text once you press return)

1.a. How was the sample size chosen to ensure adequate power to detect a pre-specified effect size?	For in vivo studies, calculations for sample size were determined using the formula: n = log / log p (Dell et al, 2002). The exact detail to determine the number of mice are described in Materials and Methods under Xenograft model and CCSP-rtTA-EGFRT790M/L858R Transgenic model sections (pgs 30 and 31)
1.b. For animal studies, include a statement about sample size estimate even if no statistical methods were used.	Statement on sample size estimation is described in Materials and Methods section (pgs 30 and 31).
2. Describe inclusion/exclusion criteria if samples or animals were excluded from the analysis. Were the criteria pre-established?	Criteria for exclusion of mice from analysis : 1) death, 2) loss of body weight of > 20% and poor well being (eg. Not eating/drinking and signs of ulcerations evident.
3. Were any steps taken to minimize the effects of subjective bias when allocating animals/samples to treatment (e.g. randomization procedure)? If yes, please describe.	Mice were randomized according to numbering - even numbered mice and odd numbered mice were allocated to respective groups. This is described in the manuscript pg 30.
For animal studies, include a statement about randomization even if no randomization was used.	Randomization statement is included in the manuscript pg 30.
4.a. Were any steps taken to minimize the effects of subjective bias during group allocation or/and when assessing results (e.g. blinding of the investigator)? If yes please describe.	The experimenters were blinded to the treatment groups, but not the investigators (non-experimenters) - as mentioned in manuscript pgs 30 and 31.
4.b. For animal studies, include a statement about blinding even if no blinding was done	The experimenters were blinded to the treatment groups, but not the investigators (non-experimenters) - as mentioned in manuscript pgs 30 and 31.
5. For every figure, are statistical tests justified as appropriate?	Yes, This was described in Materials and Methods under Statistical Analysis (pg 31). "Results from cell proliferation and viability assays, Q-PCR and FFAs quantification were from at least two independent experiments and each performed in quadruplicates, are shown as means ± s.e.m. All statistical analyses, including in vivo studies, were performed using t test and p < 0.05 was considered statistically significant. "
Do the data meet the assumptions of the tests (e.g., normal distribution)? Describe any methods used to assess it.	The t-test allows testing the null hypothesis that the population means of two groups are equal, and that the variance is assumed to be the same in both groups and is normally distributed around the group mean.
Is there an estimate of variation within each group of data?	Standard deviation or s.e.m. is provided, when applicable.
Is the variance similar between the groups that are being statistically compared?	Yes

C- Reagents

6. To show that antibodies were profiled for use in the system under study (assay and species), provide a citation, catalog number and/or clone number, supplementary information or reference to an antibody validation profile. e.g., Antibodypedia (see link list at top right), 1DegreeBio (see link list at top right).	Antibody information (ie. Vendor, catalog number and antibody dilution) is inserted in manuscript on pg 24.
7. Identify the source of cell lines and report if they were recently authenticated (e.g., by STR profiling) and tested for mycoplasma contamination.	Information on source of cell lines, authentication and mycoplasma testing is described on pg 24.

* for all hyperlinks, please see the table at the top right of the document

D- Animal Models

8. Report species, strain, gender, age of animals and genetic modification status where applicable. Please detail housing and husbandry conditions and the source of animals.	Detailed information is provided on pgs 30 and 31.
9. For experiments involving live vertebrates, include a statement of compliance with ethical regulations and identify the committee(s) approving the experiments.	Information is provided on pgs 30 and 31.
10. We recommend consulting the ARRIVE guidelines (see link list at top right) (PLoS Biol. 8(6), e1000412, 2010) to ensure that other relevant aspects of animal studies are adequately reported. See author guidelines, under 'Reporting Guidelines'. See also: NIH (see link list at top right) and MRC (see link list at top right) recommendations. Please confirm compliance.	Animal studies are reported in accordance with ARRIVE guidelines.

E- Human Subjects

11. Identify the committee(s) approving the study protocol.	N/A
12. Include a statement confirming that informed consent was obtained from all subjects and that the experiments conformed to the principles set out in the WMA Declaration of Helsinki and the Department of Health and Human Services Belmont Report.	N/A
13. For publication of patient photos, include a statement confirming that consent to publish was obtained.	N/A
14. Report any restrictions on the availability (and/or on the use) of human data or samples.	N/A
15. Report the clinical trial registration number (at ClinicalTrials.gov or equivalent), where applicable.	N/A
16. For phase II and III randomized controlled trials, please refer to the CONSORT flow diagram (see link list at top right) and submit the CONSORT checklist (see link list at top right) with your submission. See author guidelines, under 'Reporting Guidelines'. Please confirm you have submitted this list.	N/A
17. For tumor marker prognostic studies, we recommend that you follow the REMARK reporting guidelines (see link list at top right). See author guidelines, under 'Reporting Guidelines'. Please confirm you have followed these guidelines.	N/A

F- Data Accessibility

18: Provide a "Data Availability" section at the end of the Materials & Methods, listing the accession codes for data generated in this study and deposited in a public database (e.g. RNA-Seq data: Gene Expression Omnibus GSE39462, Proteomics data: PRIDE PXD000208 etc.) Please refer to our author guidelines for 'Data Deposition'. Data deposition in a public repository is mandatory for: a. Protein, DNA and RNA sequences b. Macromolecular structures c. Crystallographic data for small molecules d. Functional genomics data e. Proteomics and molecular interactions	GEO Accession Number - GSE83666 (pg 32).
19. Deposition is strongly recommended for any datasets that are central and integral to the study; please consider the journal's data policy. If no structured public repository exists for a given data type, we encourage the provision of datasets in the manuscript as a Supplementary Document (see author guidelines under 'Expanded View' or in unstructured repositories such as Dryad (see link list at top right) or Figshare (see link list at top right).	N/A
20. Access to human clinical and genomic datasets should be provided with as few restrictions as possible while respecting ethical obligations to the patients and relevant medical and legal issues. If practically possible and compatible with the individual consent agreement used in the study, such data should be deposited in one of the major public access-controlled repositories such as dbGAP (see link list at top right) or EGA (see link list at top right).	GEO Accession Number - GSE83666 (pg 32).
21. Computational models that are central and integral to a study should be shared without restrictions and provided in a machine-readable form. The relevant accession numbers or links should be provided. When possible, standardized format (SBML, CellML) should be used instead of scripts (e.g. MATLAB). Authors are strongly encouraged to follow the MIRIAM guidelines (see link list at top right) and deposit their model in a public database such as Biomodels (see link list at top right) or JWS Online (see link list at top right). If computer source code is provided with the paper, it should be deposited in a public repository or included in supplementary information.	N/A

G- Dual use research of concern

22. Could your study fall under dual use research restrictions? Please check biosecurity documents (see link list at top right) and list of select agents and toxins (APHIS/CDC) (see link list at top right). According to our biosecurity guidelines, provide a statement only if it could.	N/A