REPLY TO TRAN ET AL.:

Dimeric KRAS protein–protein interaction stabilizers

Dirk Kessler[a], Andreas Gollner[a], Michael Gmachl[a], Andreas Mantoulidis[a], Laetitia J. Martin[a], Andreas Zoephel[a], Moriz Mayer[a], David Covini[a], Silke Fischer[a], Thomas Gerstberger[a], Teresa Gmaschitz[a], Craig Goodwin[b], Peter Greb[a], Daniela Häring[a], Wolfgang Hela[a], Johann Hoffmann[a], Jale Karolyi-Oezguer[a], Petr Knesl[a], Stefan Kornigg[a], Manfred Koegl[a], Roland Kousek[a], Lyne Lamarre[a], Franziska Moser[c], Silvia Munico-Martinez[a], Christoph Peinsipp[a], Jason Phan[b], Jörg Rinnenthal[a], Jiqing Sai[b], Christian Salamon[a], Yvonne Scherbantin[a], Katharina Schipany[a], Renate Schnitzer[a], Andreas Schrenk[a], Bernadette Sharps[a], Gabriella Siszler[a], Qi Sun[b], Alex Waterson[d,e], Bernhard Wolkerstorfer[a], Markus Zeeb[c], Mark Pearson[a], Stephen W. Fesik[b,d,e], and Darryl B. McConnell[a,1]

We thank Tran et al. (1) for their appreciation of our open innovation platform (https://opnme.com/) and interest in our manuscript (2) in which we demonstrate that the small-molecule KRAS inhibitor **BI-2852** reduces pERK (EC_{50} = 6 μM) and inhibits proliferation (EC_{50} = 7 μM) in NCI-H358 cells. Tran et al. (1) propose that **BI-2852** exerts its cellular effects on the MAPK pathway at least in part through stabilization of a nonfunctional KRAS dimer. No cellular data for KRAS dimer induction are provided and the hypothesis is primarily based on KRAS dimer formation observed with size exclusion chromatography using concentrations 1,000 times higher (3 mM) than those in which the cellular effects of **BI-2852** are observed. Hence, inhibiting the protein–protein interactions between KRAS and its GEFs, GAPs, and downstream effectors is a more plausible explanation than induction of inactive KRAS dimers for the cellular mode of action for **BI-2852** (2).

However, we concur that stabilization of nonfunctional KRAS dimers as a potential therapeutic approach to treat KRAS-driven cancers is intriguing. Accordingly, we have synthesized a range of dimeric switch I/II pocket binders in analogy to linkerology (3) in the PROTAC field. We discovered compound **2**, which dimerizes KRAS with a K_D of 3.8 μM according to isothermal calorimetry measurements (Fig. 1*E*). Using our high-throughput crystal-soaking system for active $KRAS^{G12D}$ (4) (Fig. 1*C*), we obtained a dimeric KRAS structure with **2** (Fig. 1*D*) at a resolution of 1.9 Å (Fig. 1*G*), which represents the inactivating effector lobe dimer form 1 identified by Jang et al. (5) using molecular dynamics calculations. Cocrystallization of **2** with GCP-$KRAS^{G12D}$ leads to the same KRAS dimer as observed for **BI-2852** (Fig. 1 *A* and *B*), which is the second effector lobe form proposed by Jang et al. (5) at a resolution of 1.57 Å (Fig. 1*F*). In order to investigate the potential of small-molecule stabilization of nonfunctional KRAS dimers for the treatment of KRAS-driven cancers, more permeable and potent compounds will be needed to enable cellular studies.

[a]Discovery Research, Boehringer Ingelheim RCV GmbH & Co KG, 1120 Vienna, Austria; [b]Department of Biochemistry, Vanderbilt University School of Medicine, Nashville, TN 37235; [c]Discovery Research, Boehringer Ingelheim Pharma GmbH & Co KG, D-88397 Biberach an der Riss, Germany; [d]Department of Pharmacology, Vanderbilt University School of Medicine, Nashville, TN 37235; and [e]Department of Chemistry, Vanderbilt University, Nashville, TN 37235

Author contributions: D.K., A.G., M.G., A.M., L.J.M., A.Z., M.M., T. Gerstberger, M.K., J.P., J.R., R.S., A.W., M.Z., M.P., S.W.F., and D.B.M. designed research; D.K., A.G., M.G., A.M., L.J.M., A.Z., M.M., D.C., S.F., T. Gerstberger, T. Gmaschitz, C.G., P.G., D.H., W.H., J.H., J.K.-O., P.K., S.K., M.K., R.K., L.L., F.M., S.M.-M., C.P., J.P., J.R., J.S., C.S., Y.S., K.S., R.S., A.S., B.S., G.S., Q.S., A.W., B.W., and M.Z. performed research; D.C., S.F., T. Gmaschitz, C.G., P.G., D.H., W.H., J.H., J.K.-O., P.K., S.K., R.K., L.L., F.M., S.M.-M., C.P., J.S., C.S., Y.S., K.S., A.S., B.S., G.S., Q.S., and B.W. contributed new reagents/analytic tools; D.K., A.G., M.G., L.J.M., A.Z., M.M., T. Gerstberger, M.K., J.P., J.R., R.S., A.W., M.Z., M.P., S.W.F., and D.B.M. analyzed data; and D.K. and D.B.M. wrote the paper.

Competing interest statement: D.K., A.G., M.G., A.M., L.J.M., A.Z., M.M., D.C., S.F., T. Gerstberger, T. Gmaschitz, P.G., D.H., W.H., J.H., J.K.-O., P.K., S.K., M.K., R.K., L.L., F.M., S.M.-M., C.P., J.R., C.S., Y.S., K.S., R.S., A.S., B.S., G.S., B.W., M.Z., M.P., and D.B.M. were employees of Boehringer Ingelheim at the time of the work.

[1]To whom correspondence should be addressed. Email: darryl.mcconnell@boehringer-ingelheim.com.

First published February 11, 2020.

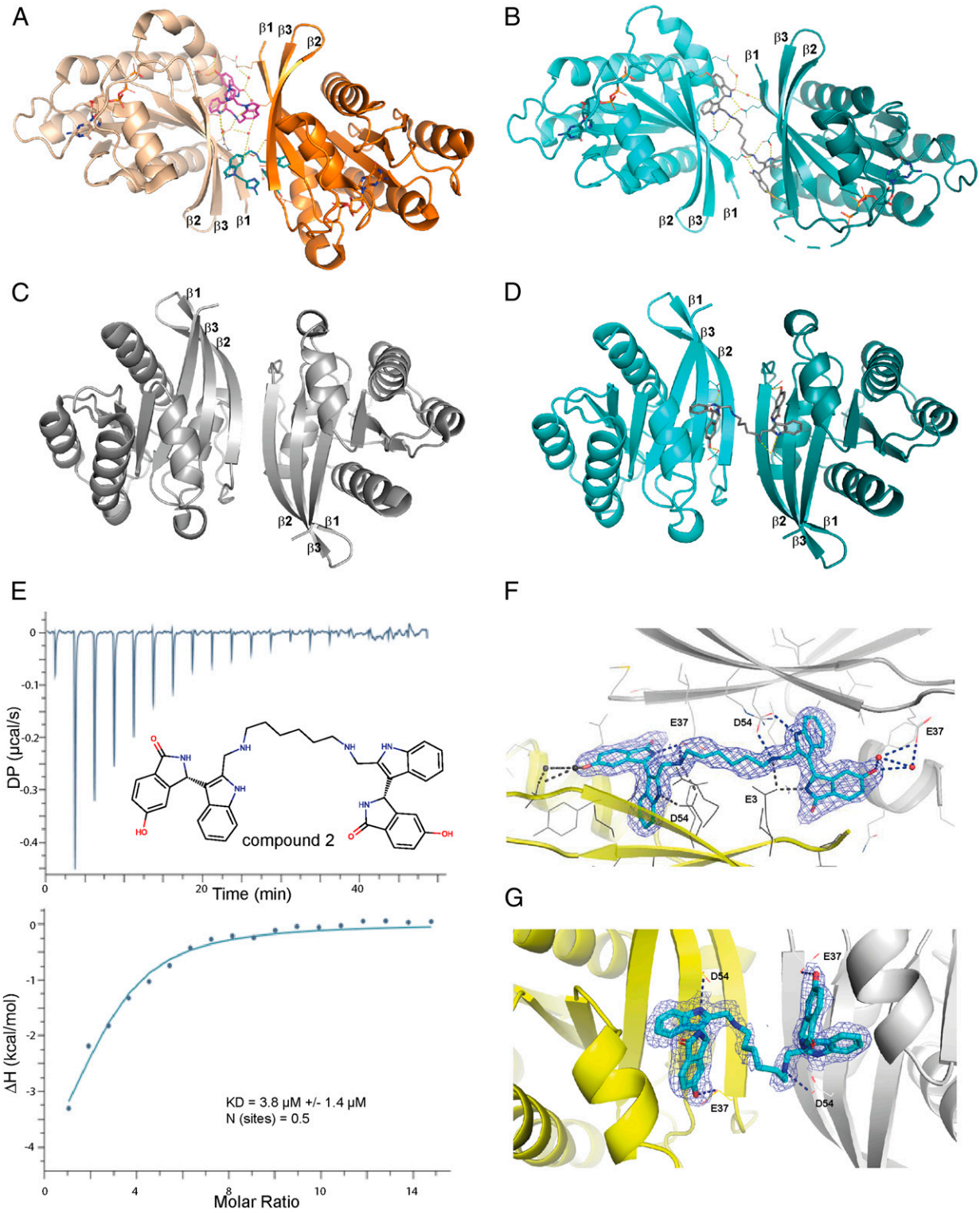

Fig. 1. KRAS dimers and biophysical validation. (*A*) KRAS G12D modeled dimer with BI-2852 (Protein Data Bank [PDB] ID code 6GJ8). The dimer interface was generated with symmetry operations from the published structure, showing a β-sandwich interface. (*B*) Cocrystal structure of KRAS G12D with compound 2 showing a similar β-sandwich interface as with BI-2852. (*C*) Published dimeric KRAS-G12D apo system (PDB ID code 6QUU) with an extended β-sheet interface. (*D*) Structure of compound 2 soaked in published KRAS-G12D soaking system. The compound needs to adapt to the extended β-sheet interface. (*E*) Isothermal titration calorimetry experiments with compound 2 showing binding to KRAS-G12D. (*F*) 2FoFc density around cocrystallized compound 2 at 1.57-Å resolution showing unambiguous binding. The interacting amino acids of both KRAS monomers are labeled. (*G*) 2FoFc density around compound 2 soaked in KRAS G12D at 1.9-Å resolution showing unambiguous binding of the ligand. The interacting amino acids of both KRAS monomers are labeled, respectively.

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