REPLY TO MARTINCORENA ET AL.:

Evidence for constrained positive selection of cancer mutations in normal skin is lacking

Benjamin D. Simons^{a,b,c,1}

In their letter (1), Martincorena et al. raise an important issue concerning the relative rates of nonsynonymous to synonymous mutations (the dN/dS ratio), and their potential implications on the survival advantage of mutant clones. Based on their original analysis (2), the authors advance possible hypotheses to explain why an apparent early survival advantage may be compatible with neutral clone dynamics, considered a paradox in ref. 3. However, although the authors are correct to highlight the fact that the dynamics of mutant clones that fall beyond the resolution limit of the deep-sequencing approach cannot be recovered, I believe that the paradox remains.

The observed collapse of the first incomplete moment of mutant clone size onto a simple exponential form is a manifestation of scaling behavior in clone dynamics and identifies just one characteristic scale: effectively, the average number of cell loss/replace-ment events of a progenitor integrated over the lifetime of the patient (3–5). “Imprisonment” of clones, as reported in studies of UVB-induced p53 mutation in mouse epidermis (6), would introduce at least one more scale, set by the typical size of the “imprisoning region.” Because the mutant clone size data are pure exponential for both synonymous and nonsynonymous mutations, showing no evidence of a second size scale, such a hypothesis would seem to be inconsistent with experiment.

As an alternative explanation, Martincorena et al. (1) point to our collaborative studies on progenitor fate in mouse esophagus following the clonal acquisition of Notch mutation, which showed that, once wild-type progenitors are purged from tissue following the

nonneutral expansion of their mutant neighbors, the resulting dynamics of the mutant population reverts back to neutrality (7). Applied to human epidermis, if a transient phase of “field expansion” occurs below the resolution limit of the deep-sequencing approach, its existence may pass undetected. However, as discussed in ref. 3, this would require all of the detectable mutant clones, synonymous and nonsynonymous, to experience precisely the same relative advantage so that neutrality of competition between adjacent mutant clones would be restored. With the clonal induction of a Notch mutation in a transgenic mouse model (5), this is the situation that prevails. However, in the context of naturally occurring point mutations, this coincidence of early advantage followed by the restoration of perfect balance seems again unlikely.

In summary, the original study of Martincorena et al. (2) is fascinating in that it provides both a quantitative window on the normal state dynamics of progenitor cell fate in human epidermis, and in its potential to expose mutation-driven field transformation (3). However, the apparent discrepancy revealed by the analysis of dN/dS ratios and the functional readout of mutant clone size remains a paradox. To gain deeper insight into whether individual heterozygous point mutations in cancer genes can indeed promote a transient survival advantage, future studies based on exome deep sequencing of physiologically normal tissues would benefit greatly from the inclusion of housekeeping genes alongside cancer drivers, so that a more objective comparison between the dynamics of mutant clones can be drawn.

- 1 Martincorena I, Jones PH, Campbell PJ (2016) Constrained positive selection on cancer mutations in normal skin. *Proc Natl Acad Sci USA* 113:E1128–E1129.
- 2 Martincorena I, et al. (2015) Tumor evolution. High burden and pervasive positive selection of somatic mutations in normal human skin. *Science* 348(6237):880–886.
- 3 Simons BD (2016) Deep sequencing as a probe of normal stem cell fate and preneoplasia in human epidermis. *Proc Natl Acad Sci USA* 113(1):128–133.

^aThe Wellcome Trust/Cancer Research UK Gurdon Institute, University of Cambridge, Cambridge CB2 1QN, United Kingdom; ^bCavendish Laboratory, Department of Physics, University of Cambridge, Cambridge CB3 0HE, United Kingdom; and ^cWellcome Trust-Medical Research Council Stem Cell Institute, University of Cambridge, Cambridge CB2 1QR, United Kingdom

Author contributions: B.D.S. wrote the paper.

The author declares no conflict of interest.

¹Email: bds10@cam.ac.uk.

- 4 Clayton E, et al. (2007) A single type of progenitor cell maintains normal epidermis. *Nature* 446(7132):185–189.
- 5 Klein AM, Simons BD (2011) Universal patterns of stem cell fate in cycling adult tissues. *Development* 138(15):3103–3111.
- 6 Zhang W, Remenyik E, Zelterman D, Brash DE, Wikonkal NM (2001) Escaping the stem cell compartment: Sustained UVB exposure allows p53-mutant keratinocytes to colonize adjacent epidermal proliferating units without incurring additional mutations. *Proc Natl Acad Sci USA* 98(24):13948–13953.
- 7 Alcolea MP, et al. (2014) Differentiation imbalance in single oesophageal progenitor cells causes clonal immortalization and field change. *Nat Cell Biol* 16(6): 615–622.