Published in final edited form as:
Nat Genet. 2018 December 1; 50(12): 1630–1633. doi:10.1038/s41588-018-0258-x.

Neutral tumor evolution?

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Competing interest
The authors declare no competing interests.

Author contribution
MT, IM, MG, AML, FM, PTS, QDM, OCL, DCW, PVL participated in argumentation. MT, OCL, DCW and PVL derived the deterministic equations. MT wrote the code and generated the figures, with input from IM, MG, OCL, DCW and PVL. MT, OCL, DCW, PVL drafted the manuscript, revised by IM, MG, AML, FM, PTS, and QDM. All authors read and approved the manuscript.

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Tumor growth is an evolutionary process governed by somatic mutation, clonal selection and random genetic drift, constrained by the co-evolution of the microenvironment1,2. Tumor subclones are subpopulations of tumor cells with a common set of mutations resulting from the expansion of a single cell during tumor development, and have been observed in a significant fraction of cancers and across multiple cancer types3. Peter Nowell proposed that tumors evolve through sequential genetic events4, whereby one cell acquires a selective advantage so that its lineage becomes predominant. According to this traditional model, the selective advantage is conferred by a small set of driver mutations, but, as the subclones that bear them expand successively, they accumulate passenger mutations as well, which can be detected in sequencing experiments1. Genomes of individual tumors contain hundreds to many thousands of these genetic variants, at a wide range of frequencies5,6. Given that genetic drift alone can drive novel variants to high frequencies, it is of great interest to discern the relative importance of selection and drift in shaping the frequency distribution of variants in any given tumor.

Williams *et al.*7 recently proposed a way to do so. They found that a simple model of tumor growth in which all novel variants are selectively neutral, that is, whose dynamics are governed entirely by drift, predicts a linear relationship between the number of mutations $M(f)$ present in a fraction f of cells and the reciprocal of that fraction: $M(f) \propto \frac{1}{f}$. They argued that deviation from this null model, i.e. the R-squared of the linear fit is below the minimum observed in neutral simulations ($R^2 < 0.98$), indicates the presence of selection and that this can be tested by means of variant allele frequencies (VAFs) from which f can be derived. Applying this rationale to real cancer data from The Cancer Genome Atlas (TCGA), the test proposed by Williams *et al.* did not reject the null model, that is neutrality, in about one third of the cases and the authors concluded that these tumors are neutrally evolving. More recently, multiple myelomas with evidence for the proposed linear relationship were associated with poorer prognosis8.

While providing an interesting approach to infer selection in human cancers, unfortunately four major simplifying assumptions underlie the analysis by Williams *et al.* that might render the conclusions questionable.

First, inferring f of variants from their VAF requires accurate estimates of local copy number, overall tumor purity and ploidy. Williams *et al.* attempted to account for some of these factors by restricting their analyses to variants with VAF between 0.12 and 0.24 and located in copy-neutral regions of the genome. However, even in that limited VAF window, the VAF of a mutation does not reflect its true f in many cases. For example, in tumors with whole genome duplications, i.e. 37% of tumors in the analyzed dataset9, the peak of clonal mutations acquired after the whole genome doubling event is at or below VAF = 0.25 (one out of four copies in a 100% pure tumor sample), which would lead to artificial deviation from the linear fit within that VAF window.

Second, the interpretation of the analyses is inconsistent with the use of neutrality as a null model. Failure to reject the null hypothesis is not the same as proving it true, i.e. that all neutral simulations have $R^2 > 0.98$ does not prove that non-neutral simulations would never yield $R^2 > 0.98$. One would need to demonstrate that this condition is sufficient to infer neutrality but also, no equally suited models of non-neutral tumor growth should yield $R^2 > 0.98$.

To assess this, we simulated simple tumor growth in which we explicitly model one subclonal expansion with a selective advantage, i.e. increasing its division rate λ and/or the mutation rate μ of the subclone (Supplementary Methods). Using the original method described by Williams *et al.*, neutrality is rejected only within a narrow range of λ and μ values tested that would lead to detectable subclones (true rejection of neutrality in ~11% of simulations; Fig. 1a). We conclude that a linear fit with $R^2 > 0.98$ is not sufficient to call neutrality and that improper use of this model could result in substantial over-calling of neutrality.

Third, the deterministic model of tumor growth described by Williams *et al.* relies on strong biological assumptions, among which are synchronous cell divisions, constant cell death and constant mutation and division rates. Stochastic models of tumor growth are biologically more realistic, as they allow for asynchronous divisions and probabilistic mutation acquisition, cell death and division rates. Using simple branching processes to simulate neutral and non-neutral growth10 (Supplementary Methods), we show that $R^2 > 0.98$ for $M(f) \propto \frac{1}{f}$ is neither a necessary nor a sufficient property of neutrally evolving tumors (Fig. 1b). Although it can be shown that the expected cumulative number of mutations – i.e. the average over many independent samples – $\overline{M}(f) \propto \frac{1}{f}$,10 due to the biological noise modeled in branching processes, a typical realization of the neutral process in a single sample deviates substantially from the expected linear fit, rendering an R-squared threshold inaccurate to infer neutrality. As a result, discrimination of neutral and non-neutral simulated tumors using a linear fit is almost arbitrary, with 53.5% false positive neutral calls in non-neutral tumors (Fig. 1b) and an area under the ROC curve of 0.42 for the classification of 1,919 neutral and 1,919 non-neutral tumors (Fig. 1c).

Fourth, we reason that in tumors called neutral, no subclonal selection should be detected. To evaluate this, we use an orthogonal method to identify selection, based on the observed variants themselves rather than on their allele frequencies. dN/dS analysis derives the

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fraction of mutated non-synonymous positions to the fraction of mutated synonymous positions in the coding regions. It has been widely used to detect the presence of negative or positive selection of non-synonymous variants in coding regions11,12. We applied a dN/dS model optimized for the detection of selection in somatic cancer variants13 to TCGA exome data using a published list of 192 cancer genes14 (Supplementary Methods). The analysis was performed separately using variants called as clonal or subclonal (Supplementary Methods), in tumors called neutral and non-neutral based on the rationale outlined by Williams and colleagues7. dN/dS ratio analysis revealed significant positive selection in subclonal mutations of tumors classified as neutral (Fig. 1d), further suggesting that the approach described by Williams *et al.* is under-equipped to detect the presence or absence of selection.

In summary, Williams *et al.* proposed that about one third of tumors are neutrally evolving. However, we highlight four simplifying assumptions – to our knowledge not previously highlighted – and find that the proposed approach will often identify individual tumors as neutral when they are non-neutral and non-neutral when they are neutral. A new paper by the same group15 introduces a Bayesian test for detecting selection from VAFs. The test estimates selection coefficients and, as such, is an important advance over Williams *et al.*'s frequentist test, which does not. The authors acknowledge that the test can only detect large fitness differences, but nevertheless call tumors that fail it "neutral" when they are merely those in which a weak test has failed to detect selection. We note that neutral theory has been developed in population genetics, ecology and cultural evolution and that similar tests have been proposed in all of these fields and, in all, eventually been found wanting for the same reason: variant abundance distributions do not contain enough information to exclude selection16–18. It is of clinical importance to identify and better understand the drivers of the potentially more aggressive (sub)clones expanding under selective biological or therapeutic pressure, as these are good candidates for predicting resistance and exploring combination therapy. Williams *et al.* are to be commended for having introduced explicit neutral tumor growth models into tumor genomics. However, quantifying the relative importance of drift and selection in shaping the allele frequencies of single tumors clearly remains an open challenge. Studies relying on their proposed test (e.g. 8) might, then, need reevaluation.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This work was supported by the Francis Crick Institute, which receives its core funding from Cancer Research UK (FC001202), the UK Medical Research Council (FC001202), and the Wellcome Trust (FC001202). MT is a postdoctoral fellow supported by the European Union's Horizon 2020 research and innovation program (Marie Skłodowska-Curie Grant Agreement No. 747852-SIOMICS). PVL is a Winton Group Leader in recognition of the Winton Charitable Foundation's support towards the establishment of The Francis Crick Institute. IM is funded by a Cancer Research UK Career Development Fellowship (C57387/A21777). DCW is funded by the Li Ka Shing foundation. This work was supported by grant 1U24CA210957 to PTS. FM would like to acknowledge the support of The University of Cambridge, Cancer Research UK and Hutchison Whampoa Limited. Parts of this work was funded by CRUK core grant C14303/A17197. This project was enabled through access to the MRC eMedLab Medical Bioinformatics infrastructure, supported by the Medical Research Council (grant number MR/L016311/1).

Parts of the results published here are based upon data generated by the TCGA Research Network: http://cancergenome.nih.gov/.

References

1. Greaves M, Maley CC. CLONAL EVOLUTION IN CANCER. Nature. 2012; 481:306–313. [PubMed: 22258609]
2. Yates LR, Campbell PJ. Evolution of the cancer genome. Nat Rev Genet. 2012; 13:795–806. [PubMed: 23044827]
3. Andor N, et al. Pan-cancer analysis of the extent and consequences of intratumor heterogeneity. Nat Med. 2016; 22:105–113. [PubMed: 26618723]
4. Nowell PC. The clonal evolution of tumor cell populations. Science. 1976; 194:23–28. [PubMed: 959840]
5. Nik-Zainal S, et al. The Life History of 21 Breast Cancers. Cell. 2012; 149:994–1007. [PubMed: 22608083]
6. Dentro SC, Wedge DC, Van Loo P. Principles of Reconstructing the Subclonal Architecture of Cancers. Cold Spring Harb Perspect Med. 2017:7.
7. Williams MJ, Werner B, Barnes CP, Graham TA, Sottoriva A. Identification of neutral tumor evolution across cancer types. Nat Genet. 2016; 48:238–244. [PubMed: 26780609]
8. Johnson DC, et al. Neutral tumor evolution in myeloma is associated with poor prognosis. Blood. 2017; 130:1639–1643. [PubMed: 28827410]
9. Zack TI, et al. Pan-cancer patterns of somatic copy number alteration. Nat Genet. 2013; 45:1134–1140. [PubMed: 24071852]
10. Bozic I, Gerold JM, Nowak MA. Quantifying Clonal and Subclonal Passenger Mutations in Cancer Evolution. PLOS Comput Biol. 2016; 12:e1004731. [PubMed: 26828429]
11. Nei M, Gojobori T. Simple methods for estimating the numbers of synonymous and nonsynonymous nucleotide substitutions. Mol Biol Evol. 1986; 3:418–426. [PubMed: 3444411]
12. Goldman N, Yang ZA. codon-based model of nucleotide substitution for protein-coding DNA sequences. Mol Biol Evol. 1994; 11:725–736. [PubMed: 7968486]
13. Martincorena I, et al. Universal Patterns of Selection in Cancer and Somatic Tissues. Cell. 2017; 171:1029–1041.e21. [PubMed: 29056346]
14. Forbes SA, et al. COSMIC: somatic cancer genetics at high-resolution. Nucleic Acids Res. 2017; 45:D777–D783. [PubMed: 27899578]
15. Williams MJ, et al. Quantification of subclonal selection in cancer from bulk sequencing data. Nat Genet. 2018; :1.doi: 10.1038/s41588-018-0128-6 [PubMed: 29273803]
16. Hammal OA, Alonso D, Etienne RS, Cornell SJ. When Can Species Abundance Data Reveal Non-neutrality? PLOS Comput Biol. 2015; 11:e1004134. [PubMed: 25793889]
17. Herzog HA, Bentley RA, Hahn MW. Random drift and large shifts in popularity of dog breeds. Proc R Soc B Biol Sci. 2004; 271:S353–S356.
18. Leigh EG. Neutral theory: a historical perspective. J Evol Biol. 2007; 20:2075–2091. [PubMed: 17956380]

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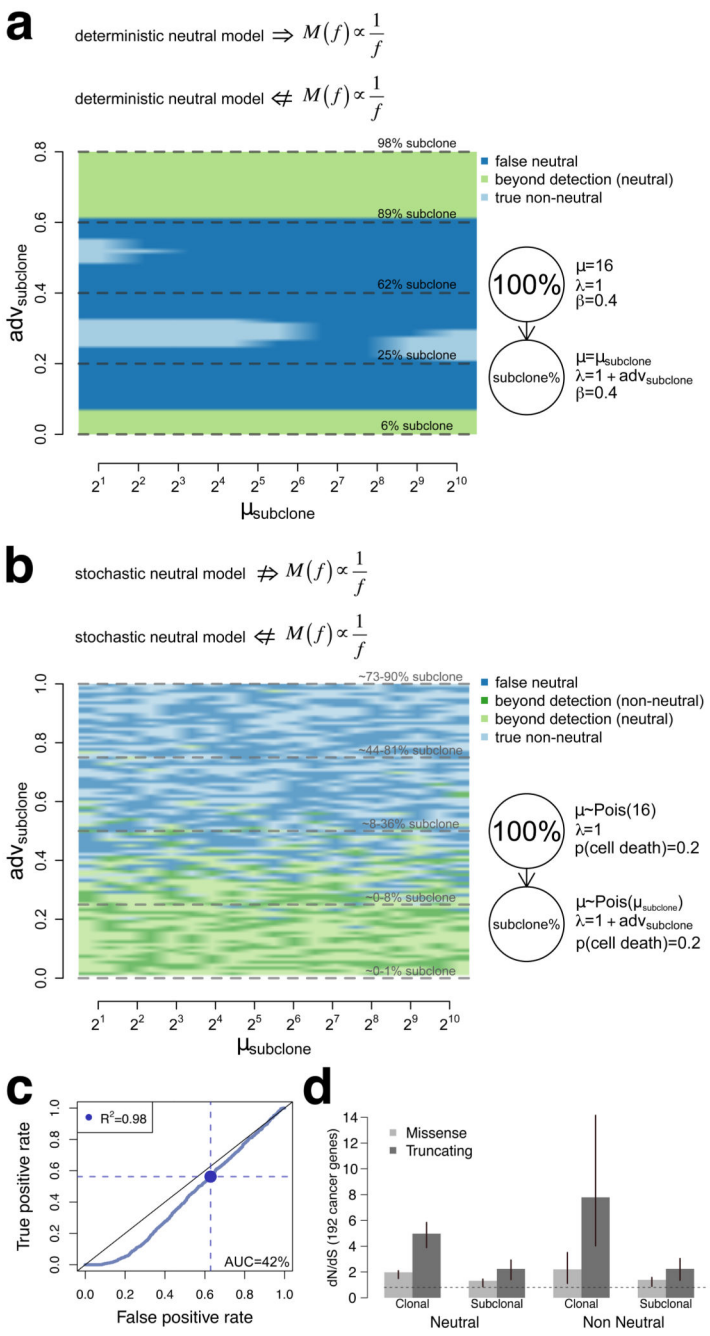

Figure 1.

(a) Neutrality calls in simulations of tumor growth with subclonal expansion underlying selective sweeps. The tree topology being modelled is represented on the right together with the parameters of the neutral evolution equations for the two subpopulations of cells (Supplementary Methods). The subclone's fraction (subclone %) increases with its selective advantage $adv_{subclone}$. We vary the $\lambda = 1 + adv_{subclone}$ and μ parameters of the subclone along a grid. Simulations are defined as true non-neutral (light blue) or false neutral (dark blue) when the growing subclone has expanded sufficiently to be detectable

and the sweep is not complete, i.e. 10% ≤ subclone % ≤ 90%, otherwise the subclone is considered beyond detection (light green). Non-neutral call: $R^2 < 0.98$; neutral call: $R^2 \geq 0.98$. **(b) As (a), using the Gillespie algorithm to simulate branching processes**10. Simulations leading to subclones beyond detection are either called neutral (light green) or non-neutral (dark green). Because of the stochastic nature of branching processes, different subclone % values are obtained across simulations from the same $adv_{subclone}$ values. For five increasing $adv_{subclone}$ values, we report median ± mad of the subclone % across the simulations. **(c) Summary ROC curve for the neutral vs. non-neutral classification based on the R^2 values in 1,919 non-neutral simulations from (b), and 1,919 simulations of neutral tumors.** The false positive rate and the true positive rate are highlighted for $R^2 = 0.98$ used by Williams *et al.* **(d) dN/dS analysis.** Maximum likelihood estimates of the dN/dS ratios and associated 95% confidence intervals for (sub)clonal mutations in TCGA tumors categorized into neutral and non-neutral groups. Ratios for missense and truncating mutations are given. dN/dS > 1 indicates positive selection.

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