Evolution, unlike development, does not follow a pre-determined pattern. Hence, each case of cancer will require the detailed treatments applied to the studies of one single species. Broad surveys can then build on many well-sampled case-by-case analyses.

While Williams et al.’s approach [4] may not be able to distinguish between neutral and selection-driven tumor growth, their analysis has been very informative about the mode of tumor growth. They found that two-thirds of cases are incompatible with the neutral exponential growth model. These cases suggest that, even at the local level far below the ‘carrying capacity’, the growth dynamics often deviates from the exponential mode that is commonly assumed. Finally, studies of tumor evolution have generally followed models developed for natural populations. A recent study [11] has proposed a more general approach to random genetic drift than the conventional Wright-Fisher model of spatial panmixia [6,7,10]. This ‘individual output’ model may be more suitable for modeling tumor evolution than practiced so far.

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On 27 April 2017, NG offered acceptance conditional on the removal of the mathematical modeling section, which makes assumptions about cell population size, N. On 2 May 2017, we replied that ‘we demonstrate the lack of power [in Williams et al.’s model] when the power is the highest possible, i.e., when N is constant. If the data used could not reject neutrality when all variables are most conducive for rejection, then the test has no power under all circumstances.’ On 4 May 2017, NG replied that keeping the mathematical modeling section would require two more months of further reviews. On 12 May 2017, this critique was withdrawn from NG. We welcome continual exchanges on a forum independent of the journal that published the original article.

We fully agree with Williams et al.’s conclusion that the neutral model should be considered the null hypothesis in the study of tumor evolution. These exchanges have hence helped to strengthen this position. We only wish to clarify the procedure of hypothesis testing. An overly simple model, such as Eq. (1) of our analysis, is useful as a gauge of the power of statistical testing because simple null models should be relatively easy to reject. For that reason, a test that fails to reject the simple model, when the model is wrong, may not have adequate statistical powers. Eq. (1) was employed because it is the simplest stochastic model with selection.

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MOLECULAR BIOLOGY & GENETICS

Reply: Is the evolution of tumors Darwinian or non-Darwinian?

Marc J Williams1,2,3,†, Benjamin Werner4,†, Chris P Barnes2,5,†, Trevor A Graham1,∗,† and Andrea Sottoriva4,∗,†

Wu and colleagues’ letter discusses the limitations of a use of a single sample per tumor to investigate neutral evolution in human cancers. Neutral tumor evolution describes the situation in which there is no differential clonal selection amongst the population of cells within a cancer: all mutations that accrue during growth are passengers and all drivers were already present in the most recent common ancestor of the population.
In Williams et al. [1], we showed, using publically available data comprising a single sample from each of ≈900 tumors of 14 different types, that the patterns of subclonal mutations within many cancer genomes (≈35%) were precisely predicted by a mathematical formula describing neutral tumor evolution. In contrast, Ling et al. [2] performed multi-region whole-exome sequencing (23 samples) and high-density targeted sequencing (286 samples) of a single hepatocellular carcinoma case and, by examining the mutation burden across the tumor, concluded that the entire malignancy was evolving neutrally.

Wu and colleagues specifically questioned whether these two different approaches, namely analysing intra-tumor heterogeneity within a sample versus dense multi-region sampling, measure the same features of tumor evolution.

Clearly, the key issue here is intra-tumor variation of the evolutionary process itself—specifically, whether some regions of a tumor are evolving neutrality and others are not. We agree with Wu and colleagues’ assertion that ‘local’ neutrality (e.g. within a single sample) does not necessarily imply ‘global’ neutrality across the whole tumor.

However, there are two reasons to think that local and global neutrality are often correlated.

First, as we discussed in Williams et al., our classifications of neutrality were consistent with the detection of subclonal driver mutations in existing multi-region sequencing studies: subclonal driver mutations and convergent evolution (consistent with ongoing selection) were often detected in ‘non-neutral-like’ renal carcinoma [3] and glioblastoma [4], but less frequently in ‘neutral-like’ colorectal cancer [5].

Second, we note that, if a single sample comprises a large portion or section across the tumor, neutrality can be assessed with our method based on the analysis of within-sample variant allele frequencies (VAF)—mutations that are subclonal within the sample. Such a large sample can provide a ‘global’ view of neutral evolutionary dynamics and, to a degree with which a single large sample represents the tumor as whole, mitigates sampling bias. A similar approach has been successfully applied to deconvolute the clonal architecture of a single breast cancer case [6]. We note that the Cancer Genome Atlas (TCGA) data we analysed in our study are derived from large fresh-frozen resection specimens rather than small biopsies (http://cancergenome.nih.gov/cancersselected/biospeccriteria), thus reducing the sampling bias of our approach. However, we fully acknowledge that no single sub-sampling strategy can fully capture the spatial architecture of a tumor and there is the need for extensive multi-region sequencing, which however remains at the moment impractical for large cohorts such as TCGA.

Importantly, as we noted in our study, the depth of sequencing remains a limitation, as it determines the time elapsed from the common ancestor (of the sampled population) where we can investigate neutral evolution, as new mutations become progressively rarer as the population grows. We agree that for low depth of sequencing, because under neutrality subclonal VAF is proportional to time, only a short period after the common ancestor can be studied and so only ‘global scale’ neutrality can be characterized, while the evolutionary dynamics of small populations remain inaccessible.

Given these two points, we think it is unlikely that our analysis risks grossly misrepresenting the tendency for neutral evolution in a tumor type.

While we fully agree that multi-region profiling reduces potential sampling bias (and indeed we use multi-region sequencing ourselves for this reason [4,5]), our method has the crucial advantage of allowing us to profile existing large cohorts (such as those of the International Cancer Genomes Consortium (ICGC) and TCGA) and so to statistically address the issue of inter-patient variation [7] within a tumor type. Clearly, the optimum would be to combine the two approaches and perform multi-region sequencing on large cohorts, though this presents obvious financial and technical challenges. Indeed, our recent study shows multiple-sample analysis of VAF distributions leads to robust calling of neutrality [8]. Moreover, we note too that studying truly ‘local’ evolution requires the sequencing of very small and localized cancer cell populations, as we previously demonstrated [5].

Wu and colleagues also note that non-exponential tumor growth leads to a different pattern of subclonal VAF in a neutrally growing tumor; boundary-driven growth (described by $N(t) \sim t^\gamma$) leads to the relationship:

$$M(f) \sim \frac{1}{f^{\gamma-1}}$$

which may provide a good fit to the data in some cases, and so neutrality may be more common than we reported in Williams et al. Irrespective, in some of the 65% of non-neutral cases identified by our method, clear subclonal mutational clusters can be observed, and our computational simulations confirmed that such patterns are expected if differentially selected subclones are present (Supplementary Figure S11 in Williams et al.—but we highlight that these clusters are not caused by boundary-driven growth. The observations of ‘subclonal clusters’ is in line with previous studies [6] and we note that, amongst the TCGA samples we re-analysed in Williams et al., a previous analysis had detected subclonal peaks in the majority of cases [9] (though we note this analysis may have confused the 1/f tail with a low-frequency clone). Thus, irrespective of the underlying growth model, there is clear evidence of ongoing selection in many tumors.

In their letter, Wu et al. describe a model of selection (Equation (1)) that predicts that the VAF distribution of a tumor sample will be indistinguishable both in the presence and absence of selection. We urge caution against using Equation (1) reported in the letter in the context of cancer, as this model appears incompatible with our current knowledge of cancer biology. First, the model assumes a constant population size, which is of course inapplicable to cancer. Second, the model also assumes that a large majority of new subclonal mutations are under selection, leading to a continuum tail of variants at higher frequency than is expected by chance...
important, this model of selection does not lead to the formation of distinct subclonal clusters in the VAF distribution \[6,10\]. We argue that such a large number of driver events at high frequency in the same cancer is highly unlikely, as most evidence points to a limited number of putative drivers per tumor \[5,11,12\]. In Williams et al., we developed a model of selection consistent with the current knowledge of cancer genomics, wherein subclones under strong selection arise infrequently during tumor growth, and where the majority of mutations are neutral passenger mutations. These dynamics do give rise to subclonal clusters of mutations, as reported by multiple studies \[6,10\]. The VAF distribution produced by these models consistently leads to rejection of the neutral null model.

However, we agree with Wu and colleagues that weak selection is challenging to detect because it causes only slight changes in the clonal composition of the tumor that may be undetectable by current genomic profiling standards. Importantly, this is true for single sampling and multi-region profiling alike, and studies of \( n = 1 \) tumors, such as that conducted by the authors \[2\], result in findings that are of uncertain generality. We acknowledge that it is very important at this stage to understand the precise signature of weak and strong selection, especially because clonal selection is often hard to define and produces complex patterns (hence one of the reasons why we focused in the original manuscript on understanding absence of selection, which is analytically tractable). This important topic is the focus of our current and future work \[13\]. Nevertheless, we note that the analysis in Williams et al. demonstrates that, in a significant proportion of cases, the null model of neutral evolution cannot be rejected through analysis of the VAF distribution.

In summary, we were very happy to see that two independent groups have now provided evidence for neutral evolution in cancer—a concept that has been largely neglected by current genomic studies. While the difference between local and global neutrality should be fully addressed in future work, the salient point that we would like people to take away from Williams et al. is that, in many cases, neutral evolution provides an entirely adequate description of the currently available data.

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