signatures reflect (and thus identify) past mutagen exposure, DNA replication/repair defects, and therapeutic response [2,3]. The high incidence of somatic mutations and the weak negative selection operating in the tumor system need to be linked to its complexity and dynamism. It has been proposed that the acquisition of supernumerary chromosomes (hyperploidy) favors oncogenesis, tumor progression, and intratumoral heterogeneity by fostering (and/or increasing tolerance to) genomic instability. While the gain of an entire chromosome or parts thereof unbalances the dosage of multiple genes, potentially including genes involved in the maintenance of genomic stability, it also buffers mutations potentially affecting essential genes [8]. Although the results on haploid regions of the tumor genome and the predominance of near-to-diploid karyotypes in neoplasms with pronounced MSI suggest that hyperploidy does not contribute to mutational burden, evolutionary genomic studies focusing on ploidy status will be necessary to formally exclude this possibility. Of note, the cancer stem cell (CSC) compartment, which is commonly viewed as being responsible for tumor initiation, evolution, and recurrence, is characterized by a robust DNA damage response [9] and heterogeneous degrees of MSI, replication stress, and chromosomal instability [10]. It will be important to investigate mutational dynamics among CSCs and how they relate with other tumor compartments in this respect.

Finally, N mutations can theoretically generate neoantigens that initiate anticancer immunity. Surprisingly, these two studies suggest that immunosurveillance operates at low levels in the course of tumor evolution. Recent evidence, however, indicates that the immunogenicity of evolving tumors is mostly shaped by neoantigen quality (rather than quantity) [4,5]. Moreover, the identification of β2 microglobulin (*B2M*) and caspase 8 (*CASP8*) as driver genes [7] suggests that defects in antigen presentation and blockade of regulated cell death may be required for cancer evolution. Thus, driver events impairing oncosuppression may be particularly advantageous to evolving tumors because they limit negative selection and potentially enable hypermutation (hence fostering tumor progression and resistance to conventional therapies). Such an advantage, however, would come with the elevated cost of increased sensitivity to immunotherapy (Figure 1).

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Spotlight

Are Lethal Alleles Too Abundant in Humans?

Mallory A. Ballinger[1] and Mohamed A.F. Noor[2,*]

Across species, many individuals carry one or more recessive lethal alleles, posing an evolutionary conundrum for their persistence. Using a population genomic approach, Amorim *et al.* studied the abundance of lethal disease-causing mutations in humans and found that, while appearing more common than expected, most may nonetheless persist at frequencies predicted by mutation–selection balance.

One might assume that mutations that cause lethality would be quickly eliminated from natural populations and thus be rarely detected. However, dozens of studies have identified or inferred alleles that cause complete or near lethality when homozygous in chromosomes sampled from fruit flies, humans, and other species. Furthermore, many, if not most, individuals of these species harbor one or more lethal alleles. Various mechanisms may contribute to the persistence of lethal alleles in a population (Table 1). Identifying recessive, lethal alleles in humans has been a major

goal in diagnosing rare, recessive Mendelian diseases.

The abundance and maintenance of lethal alleles was studied most extensively in *Drosophila* species, starting as early as the 1930s. Through a series of genetic crosses, researchers isolated single chromosomes and assessed their fitness when made homozygous. They found roughly 10–30% of such isolated autosomes were lethal or nearly so when made homozygous [1]. Lethal alleles sometimes persisted in natural populations for many generations. As a result, debates raged over the relative importance of natural selection maintaining these alleles or whether persistence simply reflects mutation–selection balance. By the 1980s, many researchers had adopted the latter perspective, citing that most individual lethal alleles are rare and that heterozygotes of lethal-bearing chromosomes tend to have reduced (rather than increased) fitness in the laboratory [2].

Human populations, too, retain such recessive lethal alleles in natural populations. For example, Gao *et al.* [3] estimated the abundance of lethal disease-causing alleles in three isolated North American populations. Using extensive genealogical records, genotype data, and simulations, they estimated that the founders of these populations had, on average, 0.58 recessive lethal alleles per diploid human genome, which is lower than other estimates [4]. They inferred that much of this burden comes from single mutations that, when homozygous, lead to sterility or death before reproductive age.

In a recent article, several of these authors have taken a population genomic approach to ask whether the observed frequency of recessive lethal alleles in humans is consistent with a simple model of mutation–selection balance [5]. In large populations, the expected equilibrium frequency of a lethal mutation is the square root of the mutation rate. In smaller populations, the expected frequency may be significantly lower. The authors started by compiling a list of known Mendelian recessive lethal diseases associated with single-nucleotide mutations. They restricted the dataset to mutations in which the homozygote was always affected and for which no effects were documented in heterozygote carriers. They then examined the frequency of these disease-associated alleles in 33 370 European humans and compared the frequencies to expectations from mutation–selection balance models. Despite several conservative assumptions in their analyses, they still found that the disease-associated alleles tended to be too abundant in European populations relative to expectations under these models, particularly for less-common types of mutations (e.g., non-CpG transversions).

Table 1. Sample Mechanisms Retaining Frequencies of Lethal Alleles in Natural Populations

Mechanism		Brief description
High genomic mutation rate		High mutation rates recurrently introducing lethal mutations, despite selection eliminating them when homozygous
Selection	Overdominance	Heterozygotes for lethal alleles have higher fitness than either homozygotes
	Pseudo-overdominance	Recessive lethal mutation is linked to one or more recessive disadvantageous mutations in repulsion – heterozygote is most fit because alternative homozygotes both carry recessive deleterious mutations
Penetrance	Epistasis	Gene interaction can cause incomplete penetrance of lethal mutations
	Environmental	Lethal mutation may have incomplete penetrance in some environments
Drive		Lethal alleles linked to driving haplotypes

While the result may seem to suggest that natural selection actively maintains these alleles in some manner (Table 1), Amorim *et al.* [5] interpreted their result differently. Since rare diseases are less likely to have been characterized, an ascertainment bias exists favoring more common Mendelian diseases, which presumably also have more common underlying variants. Hence, the results may not capture the range of frequencies of lethal alleles that exist in natural populations, with more abundant disease-causing variants being over-represented. They ran simulations and confirmed that disease alleles are more reliably represented in population samples when mutation rates are high. Their interpretation thus explains why the less-common types of mutations tended to be the most over-represented relative to expectation.

Details of the results from these two studies in humans mirror results from past research in *Drosophila*. The individual mutations studied by Amorim *et al.* [5] were not common, ranging in allele frequency from 0.001 down to 0.000015. Even summing all the distinct mutations by genes, mutations for any one gene are still at a frequency below 1% when combined. Thus, while most humans may bear one or more recessive lethal alleles, unrelated humans rarely carry the same ones. Classic findings in *Drosophila* are similar: 1% or fewer of these lethals are allelic [2], meaning the individual lethal alleles are generally rare, as predicted by mutation–selection balance.

However, understanding variation in natural populations means more than

understanding what forces affect most recessive lethal alleles, and some room exists for a minority of lethal alleles to have been influenced by distinct evolutionary forces (as acknowledged by [5]). Some lethal alleles in *Drosophila* appear unusually abundant in natural populations, either appearing at a frequency above 1% [6] or persisting for ≥8 years at a detectable frequency [7]. A few lethal alleles also exhibit heterozygote superiority as measured in the laboratory under certain conditions [1], and the allele conferring sickle-cell anemia is a classic example of overdominance in natural human populations. Surely, much of the variation in allele frequency among lethal alleles within natural populations reflects differences in mutation rates, heterozygous effects, and penetrance or genetic background, but some lethal alleles may also persist from selection facilitating their persistence (i.e., via true overdominance or pseudo-overdominance; Table 1).

Beyond humans and flies, lethal alleles are found in other species at detectable frequencies. For example, in two species of fish, McCune *et al.* [8] estimated that the average number of lethal alleles per individual is between one and two, not far from the 0.58 estimated by Gao *et al.* [3] in humans. Moreover, multiple t haplotypes in wild house mouse populations carry recessive, lethal alleles [9]. Recently, divergent male reproductive morphs in the ruff are determined by a major genomic inversion that is lethal when homozygous [10]. The plethora of genomic and phenotypic data in many species provides the opportunity to characterize variation in frequencies of lethal alleles in natural populations and understand its evolutionary roots.

The study of Amorim *et al.* [5] elegantly integrated such resources to understand lethal alleles in humans, and they were thoughtful in their interpretation of potential biases. Not only might such studies aid in better predicting disease variants, but they will also continue to advance our understanding of the forces affecting molecular variation.

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