Figure 1 Bypassing barriers on the cancer highway. Inhibition of YAP resensitizes a wide range of malignancies (cars) to BRAF and MEK inhibitors and prevents the progression of resistant lesions. The dotted line indicates potential resistance.

inhibitors targeting YAP-TEAD complexes have already been developed[13] and show some promise in animal models[14]. Importantly, this work, as well as other studies[15], emphasizes the need to develop drugs targeting master transcriptional regulators, common to a wide range of malignancies.

Finally, this report emphasizes the fact that in many cases different mutations activate similar pathways and may be treated by similar drugs, regardless of the tissue in which the tumor originated. However, the full complexities of cancer-associated genetic alterations and the intricate interactions between different players, as well as various drug resistance mechanisms, still elude us. Although the road to efficient treatment is still long, these findings are likely to encourage further research into targeting YAP in tumors and are likely to have clinical impact.

COMPETING FINANCIAL INTERESTS
The authors declare no competing financial interests.

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Avalanching mutations in biallelic mismatch repair deficiency syndrome

Joshua J Waterfall & Paul S Meltzer

Tumors from pediatric patients generally contain relatively few somatic mutations. A new study reports a striking exception in individuals in whom biallelic germline deficiency for mismatch repair is compounded by somatic loss of function in DNA proofreading polymerases, resulting in 'ultra-hypermutated' malignant brain tumors.
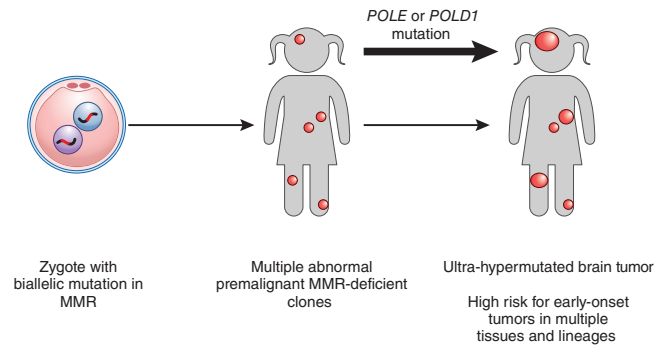
Individuals with the rare cancer predisposition syndrome biallelic mismatch-repair deficiency (bMMRD; MIM 276300) are born with loss-of-function variants of both copies of a mismatch-repair (MMR) gene (*MLH1*, *MSH2*, *MSH6* or *PMS2*)[1]. The MMR complex is a major cellular mechanism for assuring the fidelity of DNA replication, and germline MMR deficiency renders these individuals at high risk for diverse malignancies beginning at a young age. In this issue, Adam Shlien, Peter Campbell, Uri Tabori and colleagues report exome and genome sequencing of tumors from individuals with bMMRD[2]. Unexpectedly, they found that malignant brain tumors each contained a somatic mutation in one of the proofreading DNA polymerases, *POLE* or *POLD1*, leading to extraordinarily mutation-prone tumors, fittingly described as ultra-hypermutated.

Replication fidelity and cancer
In eukaryotic cells, multiple mechanisms limit the DNA replication error rate to roughly 1×10^{-10} errors per base replicated (about 1 error per cell division in humans). DNA polymerases ε and δ replicate the bulk of the genome; in addition to performing highly accurate initial incorporation, they both contain proofreading capabilities through their $3' \rightarrow 5'$ exonuclease activity. Errors that escape proofreading are subsequently repaired by the MMR complex. Inherited heterozygous deleterious variants in any one of the MMR genes

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POLE or *POLD1*
mutation

Zygote with
biallelic mutation in
MMR

Multiple abnormal
premalignant MMR-deficient
clones

Ultra-hypermutated brain tumor

High risk for early-onset
tumors in multiple
tissues and lineages

Figure 1 Mutation accumulation and cancer development in patients with bMMRD. Patients with bMMRD inherit deleterious variants in both copies of an MMR gene. Somatic mutations (rate indicated by arrow width) result in high risk for diverse cancers beginning in childhood. Malignant brain tumors have mutations of proofreading DNA polymerases, leading to mutation burdens among the highest ever documented.

cause Lynch syndrome[3], with predisposition to adult-onset cancers of the colorectum, endometrium and other organs following somatic loss of the second MMR allele. Roughly 15% of sporadic colorectal cancers are also MMR deficient, commonly because of epigenetic inactivation of *MLH1* associated with the CpG island methylator phenotype (CIMP). Inherited heterozygous variants in *POLE* and *POLD1* have also recently been shown to cause familial colorectal and endometrial cancer predisposition[4].

bMMRD is a recessive condition related to Lynch syndrome, with the difference that affected individuals inherit defects in both copies of an MMR gene[1,5,6]. These patients present with tumors of diverse organ systems, including colorectal cancers and brain tumors, in childhood[1]. Recent profiling studies of sporadic colorectal and endometrial cancers have identified subsets of these cancers with extraordinarily high mutation rates tightly associated with mutations in both MMR genes and *POLE*[7–11]. MMR-deficient tumors are hypermutated, carrying a mutation burden roughly an order of magnitude higher than that for MMR-proficient tumors. Compounding MMR deficiency with *POLE* mutations dramatically raises the mutation burden even further in these cases.

bMMRD cancer genomes

Shlien *et al.* report the whole-exome and whole-genome sequences of 17 tumors from 12 individuals with bMMRD, matched with sequence from normal DNA[2]. Strikingly, the malignant brain tumors (10 of 17) carried an extraordinarily high mutation burden (average of 249 mutations/Mb), inspiring the term ultra-hypermutated. This rate is 400-fold higher than for other pediatric cancers and is even greater than that for highly mutated tumors such as melanoma and lung cancer.

The elevated mutation rate makes it nontrivial to identify driver genes, but the authors provide compelling evidence that mutations in *POLE* and *POLD1* are causal (**Fig. 1**). Either *POLE* or *POLD1* was mutated in every ultrahypermutated tumor. These mutations clustered at conserved amino acids, and *in vitro* functional validation of a subset demonstrated a profound increase in replication errors. The variant allele frequency for these mutations furthermore suggests that they occurred early in the evolution of each tumor.

The mutation profiles in bMMRD/polymerase-mutant tumors were characteristic of the mutated polymerase. bMMRD/*POLE* tumors were dominated by C>T and C>A substitutions, and the context of the C>A mutations in particular was strongly enriched for TCT motifs, consistent with previous reports[12]. The mutation profiles for bMMRD/*POLD1* tumors were relatively enriched for T>C and T>A mutations, and the prevailing context of C>A mutations was instead CCT.

Although the ultra-hypermutated genomes had extraordinary levels of substitutions, they were stable at the structural and copy number levels, an association also seen in MMR/polymerase-mutant colorectal and endometrial cancers[7,11]. Colorectal cancers with MMR and *POLE* mutations also had stable epigenomes, being depleted for CIMP[7]. Examining whether other ultra-hypermutated cancers also show epigenomic stability would help in determining whether this is a general association. In particular, epigenomic mechanisms are significant in many pediatric brain tumors, so investigating this phenomenon in bMMRD malignant brain tumors is important.

Besides ultra-hypermutation, other diverse aspects of tumor growth must certainly be affected by the nearly 8,000 coding mutations

in each tumor. Addressing what phenotypes are affected will be essential for further understanding of the disease. Most cancer mutation profiling studies focus on positive selection, identifying somatic mutations that confer a proliferative advantage. In contrast, it is possible that the ultra-hypermutated tumors are adequately powered to detect negative selection, allowing the identification of genes that must remain unmutated to sustain tumor growth. If so, these could include novel candidate therapeutic targets.

It is also important to consider whether the ultra-hypermutated phenotype itself has clinical relevance in relation to the use of DNA-damaging chemotherapy or radiation. The remarkably reduced capabilities for error correction associated with this phenotype may accelerate the acquisition of lethal mutations in essential genes but can simultaneously abrogate important DNA damage–induced signaling pathways. Additionally, the systemic deficiency in MMR (although not proofreading) in these patients may increase risk of treatment-related toxicity[1].

As Shlien *et al.* only observed the ultra-hypermutated phenotype in malignant brain tumors, a larger series would be needed to address the true strength of this association. In particular, because the phenotype has been observed in sporadic colorectal and endometrial cancers, it is not clear why this strict tissue specificity would necessarily characterize bMMRD. Finally, whereas the mutator phenotype of these tumors may facilitate the acquisition of oncogenic mutations and their adaptability, it could also become maladaptive. Pertinent experiments in model organisms combining MMR and proofreading mutations to create mutator clones have shown that spontaneous suppressors with increased fitness do indeed arise[13]. Whether this occurs in human ultra-hypermutated tumors remains unknown.

As with many rare diseases, the interpatient consistency of ultra-hypermutated tumors arising in individuals with bMMRD provides an opportunity to improve the care of these particular patients. Because replication fidelity defects unquestionably contribute more broadly to cancer in general, investigation of bMMRD may provide a unique window into more common mechanisms.

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Big Bang and context-driven collapse

Mark Robertson-Tessi & Alexander R A Anderson

Heterogeneity is the single most important factor driving cancer progression and treatment failure, yet little is understood about how and when this heterogeneity arises. A new study shows that colorectal cancers acquire their dominant mutations early in development and that subsequent mutations, even if they confer greater fitness, are unlikely to sweep through the tumor.

Heterogeneity in cancer is an observed fact, both genetically and phenotypically[1]. Cell-to-cell variation is seen in all aspects of cancer, from early development to invasion and subsequent metastasis. This heterogeneity is also at the heart of why many cancer treatments fail, as it facilitates the emergence of drug resistance. The complex spatial and temporal process by which tumors initiate, grow and evolve is a major focus of the oncology community[2] and one that requires the integration of multiple disciplines[3]. On page 209 of this issue, Christina Curtis, Darryl Shibata and colleagues[4] illustrate perfectly the power of an integrated approach through their intriguing hypothesis that colorectal tumors grow by means of a mutational 'Big Bang'. This model is in contrast to the traditional 'clonal selection' view, where sequential mutations lead to fitter clones that sweep through the population[5]. The Big Bang model emphasizes the importance of early mutations, occurring when tumors are relatively small (10^4–10^5 cells) and environmentally proficient. Key mutations at this early stage result in growth mechanisms that are primarily tumor cell centric rather than environmentally constrained. Sottoriva *et al.*[4] do not claim that all cancers will follow this evolutionary trajectory, just a subset—specifically, those whose initial rapid growth occurs in the absence of spatial constraints. In these cases, the early proliferative clone underlies the majority of tumor growth, such that cells with subsequent (and hence private) mutations, even those resulting in greater fitness, will

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never have time to 'catch up' and sweep through the tumor. Another prediction of this work is that variegated patterns of non-localized private mutations in a Big Bang tumor indicate the presence of abnormally motile cells early in the tumor's development. Tumors with these mutation patterns, which are described as "born to be bad," have increased risk of both invasive growth and metastatic spread.

The genotype-phenotype map

An ecological view of cancer has emerged in recent years, one that explicitly considers cancer as much more than a collection of mutated cells and embraces a more dynamic dialog between tumor and host[6–8]. Critical to this view are interactions between tumor cells, between tumor and stroma, and between the tumor and its environment. Evolution and ecology are intimately entwined through mutation and selection, and both have a central role in tumor progression. However, we must understand not only the identity of the mutations that drive evolution but also the mechanisms through which these mutations manifest themselves in phenotypic change, that is, the genotype-phenotype map (**Fig. 1**). This mapping is not one to one but many to many and is fundamentally the junction at which both genes and the environment meet to produce phenotypes. Recent work has shown that the complexity inherent within the genotype-phenotype map is responsible for the difficulty in predicting evolution[9]; many genotypes produce identical phenotypes, and many phenotypes can emerge from a single genotype[10]. Indeed, recent evidence shows that genotype-phenotype mapping produces phenotypic heterogeneity through a variety of genetic and non-genetic mechanisms, including phenotypic plasticity[11], bet hedging[12], epigenetic transitions[13] and intracellular noise[14].

The study by Sottoriva *et al.* is fundamentally about the fate of the genetic mutations that drive early cancer evolution. Although the authors do not explicitly discuss the genotype-phenotype map, implicit in their results is the observation that the environment has a minor role in Big Bang tumors (**Fig. 1**). New clones arising after the establishment of the early tumor likely have little effect on the fitness of
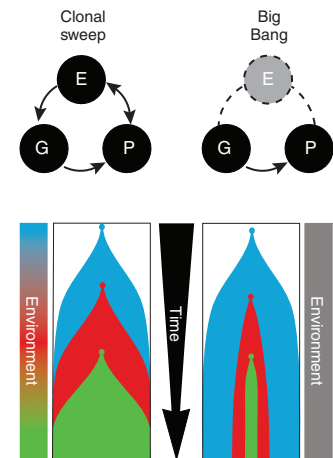
Figure 1 Clonal heterogeneity is a product of spatiotemporal evolution. The genotype-phenotype map is the dynamic manifestation of a cell's phenotype (P) based on its genotype (G) and modulated by the environmental context (E). In the traditional clonal sweep model of cancer progression (left), sequential mutations arise that take advantage of a favorable environment to outcompete the previously dominant clones. This environment may exist before the mutation, or it may be created by the mutation through feedback. In the Big Bang model of clonal growth (right), the environment has a minimal role, and fitness differences are less important than mutation timing. Early driver mutations establish a tumor that is difficult to replace through fitness advantages alone. This figure was created during a brainstorming session with Chandler Gatenbee, Jill Gallaher and Daniel Nichol, and the final version was drawn by Chandler Gatenbee.