Replication of ONYX-015 and associated CPEs and necrosis have been documented in p53-mutant tumors; adjacent normal tissue damage due to ONYX-015 has not been reported. A significant number of the p53-mutant tumor masses injected with ONYX-015 have undergone considerable destruction (40–100% reduction). Therefore, ONYX-015 has antitumoral activity in p53-mutant head and neck tumors, while not substantially harming adjacent (p53-normal) tissues after direct injection. We therefore believe that the authors' statement that "The use of adenoviruses…to target mutant p53 tumor cells may have, therefore, limited application," which was based on *in vitro* assays, is unfounded. Although preclinical testing *in vitro* and *in vivo* can be useful for hypothesis development and optimization of new agents, new mechanism-based cancer therapies must undergo rigorous, scientifically-guided clinical testing before their true value can be determined. Based on the encouraging clinical data acquired so far, we have initiated clinical trials in a number of additional tumor types using different routes of viral administration; we look forward to sharing the data from these trials as they become available.

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1. Hall, A.R. *et al.* p53-dependent cell/death apoptosis is required for a productive adenovirus infection. *Nature Med.* **4**, 1068-1072 (1998).
2. Bischoff, J. *et al.* An adenovirus mutant that replicates selectively in p53 deficient tumor cells. *Science* **274**, 373-376 (1996).
3. Heise, C. *et al.* ONYX-015, an E1B gene-attenuated adenovirus, causes tumor-specific cytolysis and antitumoral efficacy that can be augmented by standard chemotherapeutic agents. *Nature Med.* **3**, 639-645 (1997).
4. Freytag, S., Rogulski, K., Patelli, D., Gilbert, J. & Kim, J. *Hum. Gene Ther.* **9**, 1323-1333 (1998).
5. Marcellus, R.C. *et al.* Adenovirus type 5 early region 4 is responsible for E1A-induced p53-independent apoptosis. *J. Virol.* **70**, 6207-6215 (1996).
6. Kirn, D. *et al.* A phase II trial with an E1B-deleted adenovirus, ONYX-015, in patients with recurrent, refractory head and neck cancer. *Proc. Am. Soc. Clin. Oncol.* **17**, 1509 (1998).
7. Subramanian, T., Kuppuswamy, M., Gysbers, J., Mak, S. & Chinnadurai, G. The 19 kDa tumor antigen encoded by early region E1B of adenovirus 2 is required for efficient synthesis and for protection of viral DNA. *J. Biol. Chem.* **259**, 11777-11783 (1984).
8. Lane, D. Killing tumor cells with viruses-a question of specificity. *Nature Med.* **4**, 1012-1013 (1998).
9. Linke, S. Has the smart bomb been diffused? *Nature* **393**, 13-15 (1998).
10. Goodrum, F. & Ornelles, D. *J Virol.* **71**, 543-561 (1997).

Chaos in cancer?

To the editor—In his commentary on self organization, complexity and chaos in biological systems[1], Coffey states that tumors are models of chaos, whereas normal tissues are characterized by order. Although this is probably true of epithelial tumors, in mesenchyme-derived cancers, such as acute leukemias and lymphomas, quite the opposite may be found. Normal hematopoiesis and immunity are best described by chaos, with myriad clones evolving and differentiating according to a stochastic model, a characteristic that endows this system with its incredible versatility in coping with the constant variability of the antigenic spectrum. For example, the genetic instability of the variable regions of the immunoglobulin and T-cell receptor genes is the premise on which specific antibody and cell mediated immune responses are based.

In contrast, leukemia, lymphoma and myeloma cells are monoclonal, with a relative morphologic, immunophenotypic and genetic uniformity. Of course, if this homogeneity were absolute, chemotherapy results would be better that they are. However, the leukemic clone retains a certain degree of chaos, and can therefore eventually adapt to new conditions, ultimately resulting in emerging resistance. It is, however, notable that normal hematopoiesis exposed during chemotherapy to cytotoxic damage will recover in a matter of weeks (because of its versatility resulting from increased chaos), whereas the resistance of a neoplastic clone will appear in months or years. Indeed, in a small but significant percentage of patients that can actually be cured by chemotherapy, resistance may never appear.

This pattern is even more evident for malignant lymphoma; many of these patients are actually cured by cytotoxic drugs. In fact, it is a mystery why an orderly system like a hematopoietic tumor gains any growth advantage in the fist place over such a versatile, chaotic system as normal hematopoiesis. One possible explanation is an incapacity of the normal clones to deal with a certain damaging condition, resulting in a Darwinian natural selection of 'defective' clones that may eventually become malignant. For example, paroxysmal nocturnal hemoglobinuria (PNH), a clonal hematologic disease associated with increased leukemia risk, can be explained by such processes: the PNH clone has been shown to lack a set of receptors through which a subset of abnormal autoimmune cytotoxic T lymphocytes destroys the normal hematopoietic stem cells, thus offering a relative growth advantage for the PNH 'defective' cells[2]. Further clonal selection in this more orderly system (more order means less competition, therefore a better chance for a given clone to gain advantage), may lead to a premalignant myelodysplastic syndrome or to overt leukemia. As in social and political systems, controlled chaos is less likely than totalitarian order to produce the over-focused ideologies and trends that can give rise to cataclysmic events.

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Coffey replies—It is well recognized that the diversity of the immune system results from the variation in its DNA. This provides the organism with a plasticity of responses to a wide range of antigenic insults. Likewise, genetic cross-over during meiosis produces a variety of germ cells that result in variation within the population of a species, and this in turn provides a survival advantage to the species during selection. The type of genetic instability observed within immune cells and germ cells is not pathological to the organism and is indeed essential for survival. Similar types of genetic instability occur in viruses, bacteria and cancer cells, and also provide them with tremendous survival advantages. This is unfortunate when they reside within our bodies. The interplay of host, pathogens and environment is dynamic and, on a temporal basis, extremely complex. Certainly hematopoi-

etic and germ cell tumors, developing within cell populations with a high degree of biological diversity, exhibit great complexity. However, the common features of tumors—to expand a clone of cells with a growth advantage and to adapt through genetic changes—provide cancers with a survival advantage.

A systems analysis of all these biological processes reveals features similar to the interplay between order and chaos. There are many examples of complex adaptive systems in biology, such as those discussed by Andrei Cucuianu and those that I describe in my article. The complex adaptive system is a central feature of the pathology of cancer, although the strategies used to reach this complexity are different.

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1. Coffey, S.D. Self organization, complexity and chaos: The new biology for medicine. *Nature Med.* **4**, 882–885 (1998).
2. Rotoli, B. & Luzzato, L. Paroxismal nocturnal hemoglobinuria. *Sem. Hematol.* **26**, 201–207 (1989).

Fitness of antibiotic-resistant microorganisms and compensatory mutations

To the editor—The increasingly widespread use of antibiotics during the past decade has led to an alarming upsurge in antibiotic-resistant bacteria. The persistence of strains with chromosomal resistance has been observed many times. Restrictions on the use of antibiotics have been advocated not only to contain the dissemination of resistance but also to favor the disappearance of the resistant bacteria already present in human and environmental reservoirs[1–3]. Implicit in these strategies is that resistance to antibiotics reduces the fitness of bacteria (the 'cost' of resistance), allowing sensitive bacteria to replace resistant strains in a drug-free environment. In fact, it has been observed that drug-resistant bacterial pathogens with attenuated fitness rapidly accumulated various types of compensatory mutations that restored fitness without altering the level of bacterial resistance[4,5].

Several point mutations at amino acid 42 in the *rpsL* genes of *Escherichia coli* and *Salmonella typhimurium* confer resistance to high concentrations of streptomycin. The streptomycin-resistant mutants can be classified into restrictive and non-restrictive classes with respect to their translation fidelity[6]. The restrictive mutations show attenuated virulence, whereas non-restrictive resistance mutations have unaltered virulence properties, as determined experimentally in an *in vivo* model[4]. Mutants belonging to the restrictive class were used to demonstrate that compensatory mutations developed rapidly both *in vitro* and *in vivo*. The compensatory mutations increased the rate of peptide chain elongation and restored virulence[4,5]. We now provide evidence that selection for chromosomally fixed, drug-resistance-mediating mutations that confer this reduced fitness is likely to represent an *in vitro* phenomenon but may not be observed *in vivo*.

To determine which resistance mutations are found in mycobacteria, we plated strains of *Mycobacterium tuberculosis* complex and *Mycobacterium smegmatis* on agar containing streptomycin, to select for spontaneous mutations conferring drug resistance. Of 29 mutants analyzed, 16 had restrictive mutations and 13 had mutations of the non-restrictive class (Table).

Few reports directly address the cost of resistance in natural populations, and most of the evidence is indirect and anecdotal[7]. The recent emergence of drug-resistant *M. tuberculosis* provided a unique opportunity to investigate the molecular mechanisms underlying streptomycin resistance acquired *in vivo*. In *M. tuberculosis*, drug resistance is solely due to chromosomal mutations. High-level streptomycin resistance was found to be associated with mutations of codon 42 of the *rpsL* gene[8,9]. In contrast to drug-resistant mutants obtained by *in vitro* selection in the laboratory, streptomycin resistance acquired *in vivo*, with alterations of codon 42, was associated almost exclusively with mutations of the non-restrictive class [AAG (Lys) to AGG (Arg); 89 of 90 isolates investigated; Table].

The conditional character of the outcome of different selections indicates that it is important to investigate clinical isolates with drug resistance or to use relevant animal models to investigate which of the possible resistance mutations are selected under *in vivo* conditions. A high level of selection for drug-resistance mutations without involving the cost of decreased fitness would seem to exist *in vivo*. These results argue against the belief that compensatory mutations are of clinical relevance, at least for drug resistance due to mutational target alterations. Two main strategies have been proposed to alleviate the growing burden of diseases caused by drug resistant bacteria: to reduce the use of antibiotics and to develop new drugs[1,3,7]. The success of both strategies depends on a more refined understanding of the biology of antibiotic drug resistance. *In vivo* selection for resistance mutations with unaltered fitness would limit substantially the value of strategies based solely on the restrictive use of antibiotics, as these are unlikely to result in the elimination of resistant strains from reservoirs or transmission cycles. Increased knowledge and investigations of the biology of drug resistance is likely to have a considerable effect on the

Table Streptomycin-resistance mutations

	DNA sequence (amino acid) at codon 42	Resistance class
In vitro mutants[a,b]		
M. smegmatis	AAG(Lys)→AGG (Arg); *n*=11	non-restrictive
(*n*=23)	AAG(Lys)→AAC/T (Asn); *n*=9	restrictive
	AAG(Lys)→ACG (Thr); *n*=3	restrictive
M. tuberculosis complex	AAG(Lys)→AGG (Arg); *n*=2	non-restrictive
(*n*=6)	AAG(Lys)→AAC (Asn); *n*=4	restrictive
Resistance acquired *in vivo*[c]		
M. tuberculosis	AAG(Lys)→AGG (Arg); *n*=89	non-restrictive
(*n*=90)	AAG(Lys)→ACG (Thr); *n*=1	restrictive

[a]We selected spontaneous streptomycin resistant mutants from *M. smegmatis*, *M. tuberculosis H37Rv*, *M. tuberculosis* strain Erdmann and *M. bovis BCG* by plating in the presence of 50 µg/ml streptomycin; see also ref. 10.
[b]Chromosomal DNA was isolated and amplified by PCR using primers external to the coding sequence of the *rpsL* gene[8,9]. Both strands of the DNA fragment were sequenced by automated sequencing.
[c]Clinical isolates of streptomycin resistant *M. tuberculosis* (data summarized from refs. 8, 11–15).