TUMOUR HETEROGENEITY

The rise of the minority

The proportions of different subclonal populations in heterogeneous tumours are thought to vary as a result of selection pressures, which could lead to the outgrowth of subclonal variants in response to therapy. Therefore, this process of clonal evolution might promote relapse after therapy; and so understanding the dynamics of clonal evolution is an important issue and is the focus of a paper in *Cell* by Landau, Carter, Stojanov and colleagues.

To investigate genetic clonal heterogeneity in chronic lymphocytic leukaemia (CLL), the authors carried out whole-exome sequencing of CLL and matched germline DNA samples from 160 patients with CLL (for whom clinical data were also available). They then applied MuTect to the sequencing data. MuTect is an algorithm that identifies somatic mutations, including those that occur at low allelic frequencies — as would be expected of subclonal variants in heterogeneous tumours. Recurrence analysis allowed the authors to identify 20 putative CLL driver genes, nine of which were not previously known to drive CLL, and five recurrent somatic copy number alterations (CNAs) were also identified. Next, the authors used the ABSOLUTE algorithm to estimate the proportion of cancer cells that contained each of ~3,000 alterations for 149 of the 160 patient samples. This approach allowed them to identify clonal alterations (occurring in at least 95% of the sample) and subclonal alterations in each sample.

The samples from 29 of the patients who had previously been treated with chemotherapy had a significantly higher number of subclonal mutations (with no change in the number of clonal mutations) compared with samples from patients who had received chemotherapy after sample collection. Moreover, chemotherapy increased the genetic diversity of the subclones, which indicates that chemotherapy exerts a selection pressure that causes the outgrowth of subclones. Indeed, the number of driver mutations increased in subclonal populations after therapy, indicating that these cells may have a 'fitness' advantage in the presence of specific cancer therapies.

> “therapy seems to provide a selection pressure that promotes clonal evolution in CLL”

To further assess clonal evolution in CLL the authors compared the mutations in samples taken ~3.5 years apart from 18 patients. Six of these patients were not treated with chemotherapy, and the remaining 12 patients received chemotherapy in the interval between the two samples. Of the 18 sample pairs, 11 were found to have undergone clonal evolution, ten of which came from patients who had received chemotherapy, again indicating that chemotherapy may drive the expansion of subclonal populations. The authors also evaluated the effect of clonal evolution on clinical outcome. Of the ten patients who received chemotherapy and whose leukaemia exhibited clonal evolution, the time to the next occasion of chemotherapy (an indicator of symptomatic disease progression) was shortened. Furthermore, subclonal driver mutations were evident in eight of the 12 pre-therapy samples, and the corresponding eight patients also had a shortened time to next treatment, indicating that the presence of pretreatment subclonal driver mutations may correlate with a poorer prognosis. Consistently, analysis of the 149 CLL samples revealed that the presence of subclonal driver mutations was an independent risk factor for reduced time to treatment.

Therefore, therapy seems to provide a selection pressure that promotes clonal evolution in CLL, and the dominant subclones that grow out might be anticipated by identifying subclonal driver mutations in treatment-naive samples.

Gemma K. Alderton

ORIGINAL RESEARCH PAPER Landau, D. A. *et al.* Evolution and impact of subclonal mutations in chronic lymphocytic leukemia. *Cell* **152**, 714–726 (2013)
FURTHER READING Cibulskis, K. *et al.* Sensitive detection of somatic point mutations in impure and heterogeneous cancer samples. *Nature Biotech.* 10 Feb 2013 (doi:10.1038/nbt.2514)