THERAPEUTIC RESISTANCE

# ALL-important mutations

Although current treatments for acute lymphoblastic leukaemia (ALL) lead to remission in a large proportion of cases, those patients who do experience disease recurrence (approximately 20% of children and more than 50% of adults) have a poor prognosis. To understand the molecular mechanisms responsible for chemotherapy resistance and disease relapse in ALL, Meyer *et al.* and Tzoneva, Perez-Garcia, Carpenter *et al.* sequenced transcriptomes and whole exomes, respectively, of diagnosis, remission and relapse samples from patients with ALL.

There are two major subtypes of ALL — B cell ALL (B-ALL) and T cell ALL (T-ALL). Meyer *et al.* looked specifically at paediatric B-ALL, and using transcriptome sequencing of ten patients found 20 missense mutations that were present only in the relapse samples and not in the diagnosis and remission samples. Many of these were not recurrent, even on analysis of a further 62 B-ALL samples, but two different mutations in two patients were observed and validated at the DNA level in *NT5C2* (which encodes the enzyme cytosolic purine 5′-nucleotidase). Exon sequencing of *NT5C2* in an additional 61 relapse samples identified five cases with mutations, for a total frequency of seven of 71 (10%). They also used ultra-deep sequencing to identify two patients in which an *NT5C2* mutation was present as a rare subclone at diagnosis, indicating that these mutations are selected for during disease evolution. Tzoneva *et al.* sequenced whole exomes from diagnosis, remission and relapse samples taken from five patients with paediatric T-ALL and identified several relapse-specific mutations, including one in *NT5C2*. Analysis of 98 additional relapsed T-ALL and 35 relapsed B-ALL samples revealed three different recurrent *NT5C2* mutations in 19 of the T-ALLs (20 of 103, or 19% total) and one of the B-ALLs (3%). These mutations were not present in 23 T-ALL and 27 B-ALL samples taken at diagnosis.

The NT5C2 nucleotidase can inactivate the nucleoside analogues 6-mercaptopurine and 6-thioguanine, which are chemotherapeutics that are commonly used in ALL treatment. 6-mercaptopurine in particular is important for ALL maintenance therapy. Both groups tested the functional implications of the recurrent *NT5C2* mutations. Meyer *et al.* found that three of the *NT5C2* mutations they identified that led to amino acid substitutions (R238W, R367Q and S445F) increased the enzymatic activity of the mutant proteins compared with wild-type NT5C2 *in vitro*, and expression of each of these in a B-ALL cell line protected against 6-mercaptopurine- and 6-thioguanine-induced apoptosis. Tzoneva *et al.* found that the K359Q, R367Q and D407A mutations in NT5C2 increased enzymatic activity *in vitro*, and that T-ALL cell lines expressing these mutants had increased resistance to 6-mercaptopurine and 6-thioguanine. In both papers, no resistance to other chemotherapeutics was observed when the NT5C2 mutants were expressed in cell lines. Both groups also found a significant association of *NT5C2* mutations with early relapse.


> “cell lines expressing these mutants had increased resistance”

These studies emphasize the importance of looking at relapse-specific mutations in cancer genomic analyses, and could facilitate the development of more effective therapeutic regimens for ALL.

*Sarah Seton-Rogers*

**ORIGINAL RESEARCH PAPERS** Meyer, J. A. *et al.* Relapse-specific mutations in *NT5C2* in childhood acute lymphoblastic leukemia. *Nature Genet.* 3 Feb 2013 (doi:10.1038/ng.2558) | Tzoneva, G. *et al.* Activating mutations in the *NT5C2* nucleotidase gene drive chemotherapy resistance in relapsed ALL. *Nature Med.* 3 Feb 2013 (doi:10.1038/nm.3078)