NET DNA bound preferentially to laminin, the authors reasoned that NET structures facilitate proteolytic remodelling more effectively than when proteases are freely diffusible. Furthermore, the activities of NE and MMP9 exposed an epitope within laminin that in turn activated the integrin α3β1 on cancer cells to elicit a downstream signalling pathway involving FAK, ERK, MLCK and YAP leading to cancer cell proliferation.

Last, the authors engineered a blocking antibody against the NET-remodelled form of laminin; this antibody was able to inhibit the escape of cancer cells exposed to LPS and tobacco smoke in vivo.

It will be interesting to see whether these findings in mice translate to human patients, wherein a correlation can be found between chronic inflammation or smoking, the presence of NETs and cancer recurrence years after remission.

Anna Dart

ORIGINAL ARTICLE Albrengues, J. et al. Neutrophil extracellular traps produced during inflammation awaken dormant cancer cells in mice. *Science* **361**, eaao4227 (2018)

AML patients display similar alterations in IGFBP1 levels and markers of IR. As a proof of principle that this phenomenon is common to human AML, the authors showed that transplantation of a human primary leukaemia sample into immunodeficient mice recapitulates the insulin phenotype.

These findings suggest that certain cancer cells have evolved a mechanism to outcompete the host for glucose utilization that, rather than relying solely on increasing cancer cells' avidity for glucose, relies on compromising the host's normal physiology by creating a diabetic-like state. Importantly, the data also support the idea that a normal systemic glucose metabolism can have a protective effect, therefore providing a potential additional mechanistic link between metabolic syndromes and cancer.

Maria Giuseppina Baratta, Associate Editor, Nature Communications

ORIGINAL ARTICLE Ye, H. et al. Subversion of systemic glucose metabolism as a mechanism to support the growth of leukemia cells. *Cancer Cell* **34**, 659–673.e6 (2018)

LEUKAEMIA

CAR antigens beyond recognition

In clinical trials, 70–94% of patients with paediatric relapsed or refractory B cell acute lymphoblastic leukaemia (B-ALL) show complete remission in response to anti-CD19 chimeric antigen receptor (CAR) T cell therapy (CTL019). Approximately 35% of responding patients eventually relapse. Two studies, both published in *Nature Medicine*, have now reported distinct mechanisms of resistance to CAR T cell therapy, as a result of loss of CD19 surface expression.

Credit: Philip Patenall/ Springer Nature Limited

Orlando et al. analysed B-ALL samples at time of relapse from 17 patients, 12 of which had loss of surface CD19 expression. RNA sequencing and DNA sequencing of the CD19-negative samples identified mutations in CD19 in the region encoding the extracellular and transmembranal domains (exons 2–5). Mutations in this region are predicted to produce a truncated protein without membrane anchorage. There were no relapse-associated mutations in other B cell genes, and relapse was specifically associated with CD19 mutations. The allelic frequencies of these mutations were proportionate to the percentage of CD19-negative cells in the B-ALL specimens, suggesting that nearly all tumour cells in the respective samples had a CD19 loss-of-function mutation. Furthermore, B cell populations in relapse patients were almost entirely CD19-negative, and loss of heterozygosity occurred in most of those patients. Other mechanisms of CD19 loss, such as alternative splicing or lineage switching, were excluded.

Ruella, Xu, Barrett et al. report the mechanism for relapse of a B-ALL patient, who, in frank relapse at around 9 months following CAR T cell therapy, had bone marrow infiltration of CD19-negative leukaemic cells as well as circulating CAR-transduced B cell leukaemia cells (CARB cells), and eventually died. Gene rearrangement analysis showed that CARB cells were clonally related to the original leukaemia, and thus the authors investigated whether relapse was due to lentiviral transduction occurring in vivo or during the CTL019 manufacturing process. The latter was confirmed to be the case. As such, the number of vector integration sites fell from 2,924 sites in the CTL019 infusion product to two integration sites comprising 97% of all sites sampled at time of relapse, indicating that transduced leukaemic blasts clonally expanded, leading to leukaemia relapse. Furthermore, this patient relapsed with CD19-negative leukaemia. Relapsed B-ALL blasts expressed CAR19 and CD19, which were colocalized on the cell surface, as detected by confocal microscopy. However, CD19 could not be detected by antibodies targeting its extracellular domain. This was due to CAR19 masking the CD19 epitope through binding to CD19 on the surface in *cis*, which mediated CD19-specific resistance of this clone to CTL019. Similarly, in vitro analysis showed that transduction of a B-ALL cell line with a CD22 CAR confers specific resistance to CD22 CAR and not to CD19 CAR. However, it remains unclear how unintended leukaemic cell transduction can be avoided during the CTL019 manufacturing process. Nonetheless, retrospective analysis of relapsed patients showed that the frequency of CD19 epitope masking in patients was very low, and none of the other relapsed patients had CAR19 expression in leukaemic cells.

Patients with relapsed CD19-negative leukaemia, in which CD19 surface expression is lost, might benefit from CAR T cell therapy targeting alternative antigens. However, the improvement of manufacturing techniques is required to minimize the risk of introducing CAR-transduced leukaemic cells that could confer specific resistance to therapy.

Ulrike Harjes

ORIGINAL ARTICLES Orlando, E. J. et al. Genetic mechanisms of target antigen loss in CAR19 therapy of acute lymphoblastic leukemia. *Nat. Med.* **24**, 1504–1506 (2018) | Ruella, M., Xu, J., Barrett, D. M. et al. Induction of resistance to chimeric antigen receptor T cell therapy by transduction of a single leukemic B cell. *Nat. Med.* **24**, 1499–1503 (2018)