Recurrent histone mutations in T-cell acute lymphoblastic leukaemia

Mutations affecting key modifiable histone type 3 (H3; Table SI) residues are frequent oncogenic events in certain solid tumours (Feinberg et al, 2016), and have also recently been implicated in a subset of acute myeloid leukaemia (AML) (Lehnertz et al, 2017). Here, we systematically reviewed the somatic mutations in >20 000 cancer specimens to identify tumours harbouring H3 mutations. In a subset of T-cell acute lymphoblastic leukaemia (T-ALL) we identified non-methionine mutations of the key modifiable H3 residues, lysine (K) 27 and 36.

The starting point of our investigation was a search for H3 hotspot mutations in 1020 human cancer cell lines (Table SII). In two cell lines, both derived from T-ALL, we found lysine-to-arginine mutations at H3K27 and H3K36 (Table I). One of the cell lines, LOUCY, is derived from a T-lymphoblastic blast crisis of chronic myeloid leukaemia (Kuriyama et al, 1989). Ten further T-ALL cell lines lacked coding H3 mutations (Table SIII). In solid tumours, H3K27 and H3K36 are typically mutated to methionine (Fig 1) (Feinberg et al, 2016). However, recent functional studies of H3 lysine-to-isoleucine mutations in AML demonstrate that the latter also dramatically alter global H3 methylation and acetylation patterns (Lehnertz et al, 2017). Therefore, we speculated that lysine-to-non-methionine mutations may also be drivers of a subset of T-ALL.

We next searched for canonical H3 mutations in a published targeted sequencing study of 633 epigenetic regulator genes in >1000 childhood tumours encompassing 21 cancer subtypes (Huether et al, 2014). Amongst 91 T-ALL specimens, there were two cases with canonical H3 mutations: H3F3A p.K27R and H3F3A p.K36R (Table I). Both mutations were clonal, with a variant allele fraction (VAF) of 38% and 55%, respectively. Among the 37 tumours with H3K mutations, lysine-to-arginine mutations were restricted to T-ALL (P = 0·001502; Fisher’s exact test).

We then extended our screen for H3 mutations to 18 704 tumours, encompassing >60 cancer types other than T-ALL.

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(Tables SIV and SV). This dataset comprised 8764 internally sequenced specimens and 9940 TCGA samples re-analysed using an in-house variant calling pipeline as previously described (Martincorena et al., 2017). We identified only one neomorphic H3 mutation in an acute leukaemia specimen: a previously reported HIST1H3D p.K27M mutation in an adult AML case (TCGA-AB2927-03) (Lehnertz et al., 2017).

Finally, we examined an additional T-ALL cohort by capillary sequencing of recurrently mutated modifiable residues K27 or K36. CML, chronic myeloid leukaemia; ETP-ALL, early T cell precursor acute lymphoblastic leukaemia; T-ALL, T cell acute lymphoblastic leukaemia.

### Table I. Type 3 histone mutations in T cell leukaemia.

| Sample name | Sample type | Donor age (years) | Donor sex | H3 mutation     |
|-------------|-------------|-------------------|-----------|-----------------|
| LOUCY       | Cell line derived from ETP-ALL | 38            | Female    | HIST1H3G p.K36R |
| CML-T1      | Cell line derived from the acute T-lymphoblastic blast crisis of CML | 36            | Female    | H3F3A p.K27R    |
| SJTALL174   | Primary ETP-ALL specimen | Unknown (paediatric) | Unknown | H3F3A p.K36R    |
| SJTALL080   | Primary T-ALL specimen | Unknown (paediatric) | Unknown | H3F3A p.K27R    |
| PD2752a     | Primary T-ALL specimen | 30            | Male      | H3F3A p.K27N    |

Out of 141 T cell leukaemia specimens screened (12 cell lines and 129 primary samples), 5 (3.5%) harboured a missense mutation at a modifiable lysine residues K27 or K36. CML, chronic myeloid leukaemia; ETP-ALL, early T cell precursor acute lymphoblastic leukaemia; T-ALL, T cell acute lymphoblastic leukaemia.

### Fig 1. Prevalence and amino acid specificity of type 3 histone mutations in different cancer types. Columns indicate cancer types and rows show key histone type 3 regulatory residues. Tiles are coloured according to amino acid substitution. The percentage of each tumour type affected by the given class of histone mutation is indicated within the tiles and the overall prevalence of histone mutations is summarised at the bottom of each column. NBS HGG, non-brain stem high grade glioma; DIPG, diffuse intrinsic pontine glioma; ASTR, astrocytoma; AML, acute myeloid leukaemia; T-ALL, T cell acute lymphoblastic leukaemia; OS, osteosarcoma; ADM, adamantinoma; GCTB, giant cell tumour of bone; CCC, clear cell chondrosarcoma; CB, chondroblastoma; CS, chondrosarcoma.

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A feature of H3 mutations in solid cancers is their exquisite tumour type specificity (Fig 1) (Feinberg et al., 2016). In this context, it is notable that 5/5 H3 mutations in T-ALL identified by this survey are lysine-to-non-methionine mutations, and 4/5 are lysine-to-arginine mutations. Out of the >20 000 tumour specimens screened for H3 variants, only two other samples harboured H3 lysine-to-arginine mutations, both at low VAF and in tumours with relatively high coding mutation burdens (TCGA-BT-A20Q-01 and TCGA-AN-A0FW-01). Hence, it is possible that lysine-to-arginine mutations confer particular selective advantage in the context of T cell leukaemogenesis.

In summary, ~3% of T-ALL harbour non-methionine variants in H3 genes at key modifiable lysine residues. Given the role of dysregulated H3K27/H3K36 modification in T-ALL pathogenesis and the established prognostic significance of mutations in lysine-specific histone modifiers (Belver & Ferrando, 2016), this finding warrants further investigation of the prevalence, clinical and functional significance of H3 mutations in T-ALL. In light of the recent discovery of oncogenic H3K37 mutations in AML (Lehnertz et al., 2017), our findings suggest a broader role for histone mutations in acute leukaemias and clearly justify incorporation of H3 genes into haematological cancer sequencing panels.

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Authorship
S.B., M.R.S. and P.J.C. conceived and designed the study. G.C. and S.B. performed analysis with input from M.Y., I.M. and N.B. L.F. contributed materials. G.C. and S.B. wrote the manuscript with contributions from G.S.V. and P.J.C.

Conflict of interest
The authors have no competing financial interests to declare.

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Bortezomib plus EPOCH is effective as frontline treatment in patients with plasmablastic lymphoma

Plasmablastic lymphoma (PBL) is a rare and aggressive CD20-negative lymphoma associated with poor outcomes. Multiple studies have shown median survival times of 12–18 months (Castillo et al, 2012; Schommers et al, 2013; Morscio et al, 2014). Several case reports and small case series have suggested an increased response rate in patients treated with bortezomib alone or in combination, especially the combination of bortezomib and dose-adjusted etoposide, prednisone, vincristine, cyclophosphamide and doxorubicin (V-EPOCH) (Castillo et al, 2015a; Fedele et al, 2016). Due to its rarity, prospective studies exclusively in PBL patients are unlikely to be performed. We evaluated the potential therapeutic value of V-EPOCH in patients with PBL.

We retrospectively reviewed medical records at participating institutions of all patients with a diagnosis of PBL who received frontline V-EPOCH. The lymphomas were required to have plasmablastic morphology, lack CD20 expression and to its rarity, prospective studies exclusively in PBL patients are unlikely to be performed. We evaluated the potential therapeutic value of V-EPOCH in patients with PBL.

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