**SUPPLEMENTARY APPENDIX**

# Supplementary Data

* **Supplementary Discussion**
* **Supplementary References**
* **Supplementary Tables 1-3 (separate file)**
* **Supplementary Figures 2 (separate file) and legend**

**SUPPLEMENTARY DATA**

**Detailed clinical history of Case 1 developing cHL and AML**

* May 2013: diagnosis of nodular sclerosis classic Hodgkin Lymphoma, stage IVB, EBV-negative.
* May 2013 – May 2014: As first-line therapy for cHL, the patient received 5 cycles of ABVD regimen (doxorubicin: 25 mg/m2, days 1, 15; bleomycin: 10 mg/m2, days 1, 15; vinblastine: 6 mg/m2, days 1, 15; dacarbazine: 375 mg/m2, days 1, 15)1. PET- CT carried out after two cycles documented partial remission; the fifth cycle was only partially administered due to stable disease revealed by PET-CT performed at the end of the fourth cycle. The patient then received 3 cycles of IGEV salvage regimen2 (ifosfamide 2000 mg/m2 on days 1 to 4; gemcitabine 800 mg/m2 on days 1 and 4; vinorelbine 20 mg/m2 on day 1; prednisolone 100 mg on days 1 to 4), followed by collection of 10.9x106/Kg CD34+ peripheral blood hematopoietic stem and progenitor cells (HSPCs) mobilized with G-CSF. The patient underwent autologous hematopoietic stem cell transplantation (ASCT) of 5.45x106/Kg CD34+ HSPCs following the FEAM conditioning regimen (fotemustine 150 mg/m2 on days - 7, -6, etoposide 200 mg/m2 and cytarabine 400 mg/m2 on days -5, - 4, -3, -2; melphalan 140 mg/m2 on day -1)3. G-CSF was administered at day +6 post- infusion and engraftment occurred at day +8 post-infusion. Toxicity included: grade 2 mucositis and an infectious episode at day +4. Mediastinal mass radiotherapy (total dose of 40 Gy) was performed after ASCT as consolidation. The patient achieved a complete remission of cHL by PET-CT, which was durable in the subsequent follow-up.
* February 2019: diagnosis of AML with normal karyotype carrying *NPM1*mut and

*FLT3*ITD mutations, including a leukemia cutis presentation.

February – July 2019: AML induction therapy with “7+3” regimen (cytarabine 100 mg/m2 on days 1 to 7; daunorubicin 60 mg/m2 days 1, 2, 3). A complete remission was achieved. A consolidation course with high-dose cytarabine (cytarabine 1.5 g/m2 days 1, 3 and 5) plus midostaurin (50 mg orally twice daily, on days 8 through 21)4 was then administered. Leukemia cutis relapsed about one month later and, despite subsequent treatment with etoposide (100 mg/m2, from days 1 to 7) and high-dose cytarabine (3 g/m2, days 1,3, 5, 7), leukemic progression occurred followed by death shortly after.

# SUPPLEMENTARY DISCUSSION

**First description of a human B-cell lymphoma harboring a *DNMT3*A-R882 mutation** cHL Case 1 represents, to the best of our knowledge, the first description of a *DNMT3A*R882 hotspot mutation in tumor cells of a human B-cell lymphoma. In individuals with CHIP and in patients with AML, *DNMT3A* mutations at codon 882, which are the most prevalent *DNMT3A* mutations and likely result in loss-of-function5-6, have been found in all blood cell lineages, including B cells, and are thus considered to occur in multipotent stem cells5-9. In mouse models conditionally knocking-out *Dnmt3a*10-14 or knocking-in *Dnmt3a*R878H (the murine homologue to human *DNMT3A*R882H)15,16 in hematopoietic stem/progenitor cells, some biased differentiation potential through the B-cell lineage10 and the development of mature B-cell tumors13,14 have been reported. However, in humans, mutations of *DNMT3A* were very rarely reported in tumor cells of B-cell neoplasms, for example at frequencies <1%17 and <3%18 in diffuse large B-cell lymphoma (DLBCL) studied by WES (including 526/574 and 601/1001 cases, respectively, analyzed in the absence of matched non-neoplastic cells, whose sequencing could prevent the calling of CHIP-associated mutations). Notably, none of these rare *DNMT3A* mutations involved the R882 hotspot17,18, as instead we found in both the HRS cell and reactive tissue components of EBV+ cHL Case 1 with an otherwise almost null exome-wide somatic mutation burden (known to be low in EBV+ cHL19). However, whether or not *DNMT3A*R882 mutations may play a role in (EBV+) cHL pathogenesis, similar to *TET2* mutations in DLBCL pathogenesis20, requires additional investigations.

# Post-therapy AML arising from a large pre-existing CH clone

Among myeloid neoplasms arising from CHIP after cytotoxic treatments, including ASCT for lymphoma, it is important to recognize the genetically distinct cases of *de novo* AML carrying *NPM1* mutations and normal karyotype (or anyway lacking therapy-related

cytogenetic abnormalities) that can arise from a large pre-existing CHIP clone driven by *DNMT3A*, *TET2* and/or *ASXL1* mutations (this report and refs. 21, 22, 23). Such *de novo* AML may develop in a therapy-unrelated manner that depend just on the higher risk of neoplastic evolution intrinsic to a CHIP clone already considerably expanded (refs. 22, 24 and this report); or in a manner that may be influenced by the previous treatment but through mechanisms distinct from those at play in the more frequent and typical cases of therapy-related myeloid neoplasms. Indeed, the latter usually carry an abnormal karyotype (often complex and/or including loss of chromosome 5 and 7) and evolve from a frequently less extensive CHIP sustained by mutations disabling *TP53* and/or *PPM1D*, which confer resistance to cytotoxic treatments and are therefore often directly selected by therapy23,25-28. Although myeloablation during ASCT can promote the growth of pre-existing CHIP clones due to their higher fitness in reconstituting hematopoiesis29, this phenomenon unlikely promoted AML development in our cHL patient (Case 1), as his CH was already massive before transplant. Anyway, further studies on larger number of cases are needed to better understand the specific pathogenetic mechanisms underlying the development of such atypical post-therapy myeloid neoplasms.

Finally, it is interesting to note that, in Case 1, AML developed from a massive CH (VAF 47%) 6 years after CH was identified in coincidence with cHL diagnosis at 45 years of age. Conversely, in another patient we recently described22, AML developed just one year after identifying at the same age a similarly massive CH (VAF 49%) and an angioimmunoblastic T-cell lymphoma evolved from it. Such different lag time in AML development in these two patients, despite the very similar CH size identified at the same age, could be due at least in part to the fact that in the former case only one gene mutation underlay CH (*DNMT3A*R882H), whereas in the latter case the CH clone originating AML had multiple mutations disrupting two genes22, 24, 30 (*TET2*, likely in a biallelic manner, and *ASXL1*22).

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