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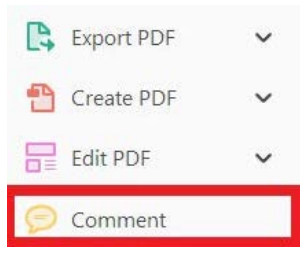

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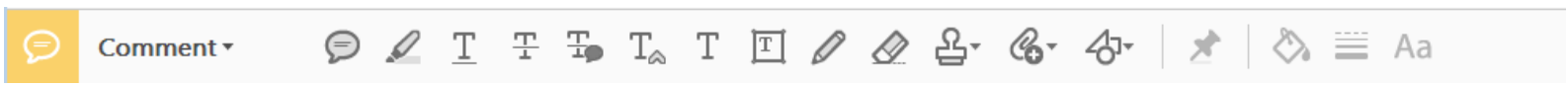

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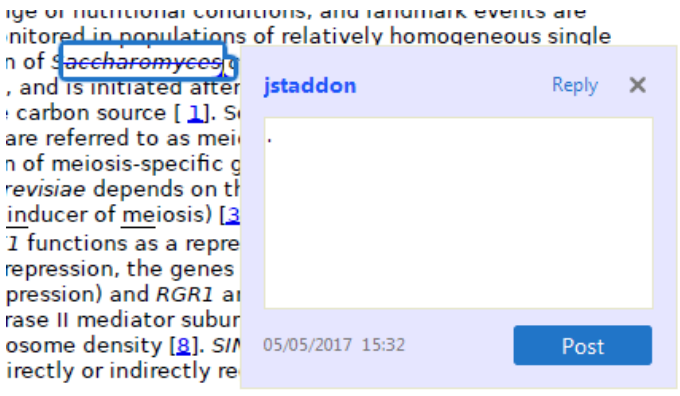

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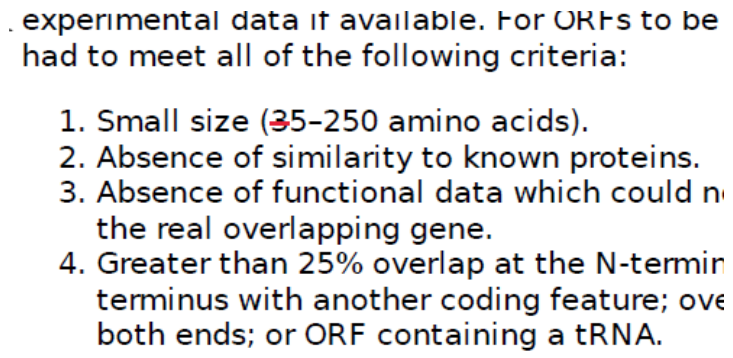
experimental data if available. For ORFs to be had to meet all of the following criteria:

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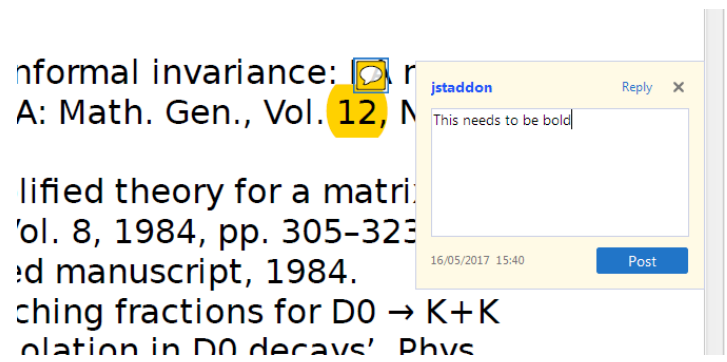

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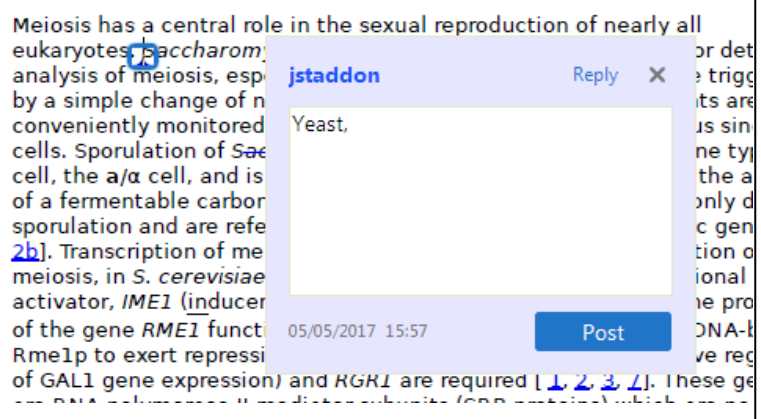

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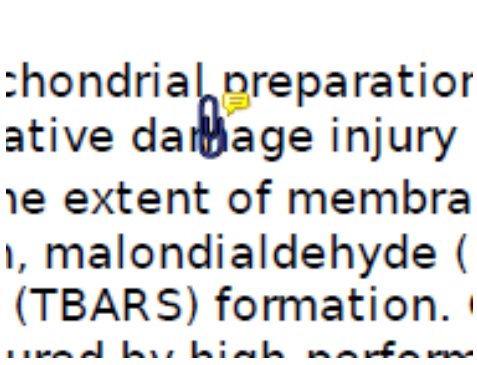

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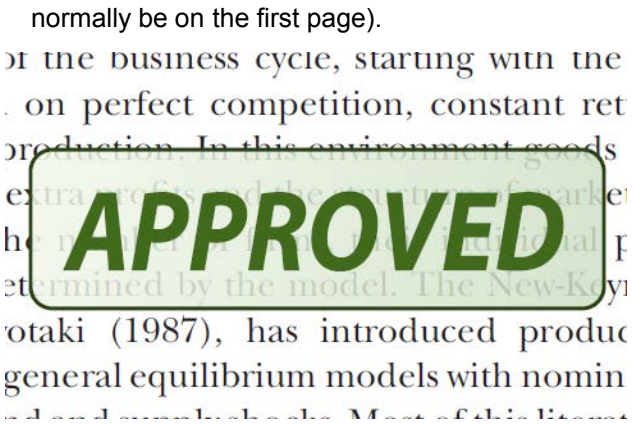

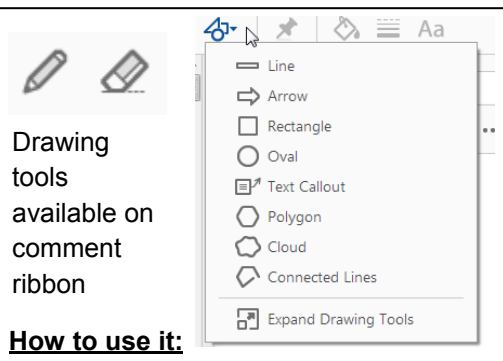

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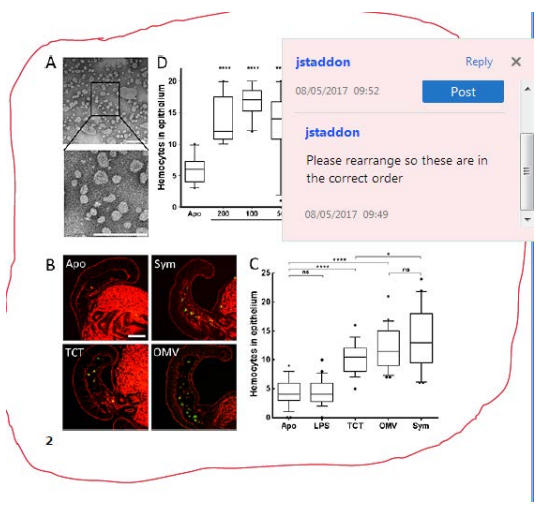

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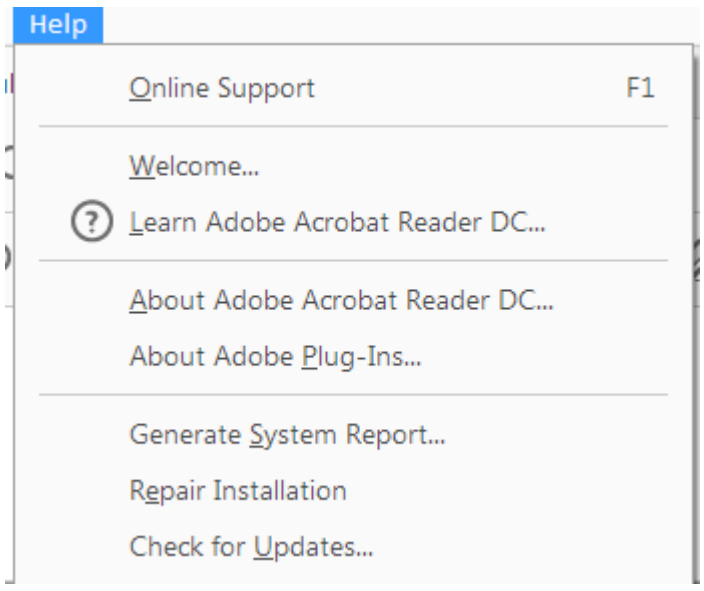

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A blastic plasmacytoid dendritic cell neoplasm-like phenotype identifies a subgroup of npm1-mutated AML patients with worse prognosis

To the Editor:

As widely reported, isolated *NPM1* mutations display a positive prognostic value in acute myeloid leukemia (AML) since they are associated with high complete response rate to chemotherapy and with reduced relapse risk, especially if a molecular complete remission (mCR) is achieved.[1] However, a minority of NPM-mut AML patients do not achieve hematological or mCR or display early relapse, irrespectively of FLT3 status. Despite its recognition as a distinct WHO 2016 entity, NPM-mut AML displays indeed a certain degree of clinical and biological heterogeneity. Morphologic spectrum is wide and can involve all the FAB subtypes, with the exception of M3, with blasts frequently showing monocytic differentiation and cup-like nuclei. Even immunophenotype (IF) is not univocal; NPM-mut cells are usually CD34 negative, CD33 and CD13 positive and a "myeloid" or "monocytic" IF can be usually distinguished. No prognostic relevance has been associated to morphological and immunophenotypic features so far.

We retrospectively evaluated 38 consecutive young, de novo NPM-mut AML patients diagnosed in our institution between 2006 and 2014 and treated with a fludarabine, high dose cytarabine and idarubicin (FLAI) based induction.[2] Multicolor cytofluorimetric analysis was routinely performed on bone marrow samples obtained at diagnosis, to define lineage according to WHO 2016, and to identify the leukemia associated phenotypes for minimal residual disease (MRD) monitoring. MRD assessment was performed in all patients with both IF and RQ-PCR for *NPM1* expression levels quantification, after induction and each of the consolidation courses. In our experience, a greater than 3.5 logarithmic reduction of *NPM1* expression after FLAI induction identified patients with the best probability to achieve mCR and best long term outcome.

The retrospective review of leukemic immunophenotypes at diagnosis allowed us to identify three different subgroups of patients; 16/38 displayed a myeloid IF [CD33/CD13/CD38/CD117/MPO (+)]; 7/38 a monocytic IF [CD33/CD64/cyLys/CD11b/CD15 (+) with 3/7 patients CD13+]; the third group included 10 patients who displayed both myeloid, and monocytic features [CD33/CD13/CD38/CD117/MPO/CD64/cLys/CD11b/CD15 (+)]. Five patients could not be assigned to any of those groups.

FLT3-ITD mutation was detected in 16/38 (42%) patients. Its incidence was significantly higher in the monocytic group, however this did not translate in a worse outcome (data not shown). No statistically significant differences in relapse free survival (RFS) and overall survival (OS) were detectable among the three IF groups. The expression of CD34 did not negatively affect RFS and OS. Interestingly, searching for recurrent aberrant antigen combinations, we identified six patients with [CD56/CD123/CD4 (+)] coexpression; in other seven patients only two of these three markers were present. Since these markers represent part of the typical blastic plasmacytoid dendritic cell neoplasm (BPDCN) IF, we named this phenotype "BPDCN-like". BPDCN-like IF was equally distributed among the previously described IF subgroups. Three out of the 6 BPDCN-like patients displayed concomitant FLT3 ITD mutation and all patients had normal karyotype. None of these BPDCN-like patients displayed clinical, morphological and biological features generally associated with BPDCN.[3] Overall, the outcome of BPDCN-like patients was poorer compared to those not expressing this antigen combination. Specifically, five out of six BPDCN-like patients achieved CR after induction (83%) but only one patient achieved mCR. Allogeneic stem cell transplantation (HSCT) was scheduled for refractory patients and for those not achieving mCR, with three patients being transplanted. Three out of five patients not obtaining mCR could not be transplanted due to a sudden unresponsive disease relapse. A complete overview of BPDCN-like patients' features at diagnosis, response to treatment, and long-term outcome is provided in Table 1. Three year RFS was 28 and 72%, respectively, for patient with or without BPDCN-like phenotype ($P < .05$), whereas 3-year OS was 0 and 63%, respectively ($P < 0.05$). Furthermore, a trend towards an inferior OS was observed even in the seven patients presenting only two of three BPDCN markers. Although the negative impact of each of these antigens has already been described, to the best of our knowledge this is the first report on the prognostic impact of CD123, CD56, and CD4 coexpression in NPM mut AML. CD123 is strongly expressed by plasmacytoid dendritic cells and by their pathological counterpart in BPDCN and it is widely expressed in hematological malignancies. It is also expressed on physiological CD34+ hemopoietic progenitors and on leukemic AML stem cells (LSC). The number of CD123+ LSC has been shown to be predictive of clinical outcome. Interestingly, a negative prognostic impact of CD123 expression in NPM-mut AML has already been reported.[4] A negative prognostic impact for CD56 expression in AML has been reported, especially for AML with t(8;21) and acute promyelocitic leukemia[5] and the expression of CD4 has been recently associated to an unfavorable outcome in wild-type *NPM1*, *FLT3*-ITD-negative cytogenetically normal AML.[6]

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TABLE 1 Overview of NPM-mut AML and BPDCN like patients; features at diagnosis, response to treatment, MRD assessment, and long-term outcome

All NPM-MUT AML patients									
Number of patients	Median age	FLT3 ITD	Response to FLAI induction	Post FLAI NPM log red > 3.5	mCR	HSCT	Relapse	DFS	OS
38	48	16 (42%)	36 CR	26 YES	28 YES	28 NO	31 NO	Mean 56	Mean79 Median NR
			1 NR	10 NO	8 NO	10 YES	7 YES	Median NR	
			1 ED	2 NV	2 NV				

NPM-MUT AML patients with BPDCN-LIKE IF											
Patient	Age	FLT3 status	Response to FLAI induction	Post-FLAI NPM1 log red > 3.5	mCR	Status at HSCT	HSCT	Relapse	DFS	OS	Alive
#1	57	ITD[b]	CR	NO	NO	NA	NO	YES	9	14	NO
#2	50	Wt	CR	NO	NO	NA	NO	YES	4	6	NO
#3	35	ITD^{high}	CR	NO	NO	NA	NO	YES	5	6	NO
#4	45	Wt	CR	YES	NO	CR1	YES	NO	25	26	YES
#5	55	Wt	CR	YES	YES	CR1§	YES	NO	36	37	NO
#6	38	ITD^{high}	NR	NO	NO	REF	YES	NA	0	7	NO

[a]Pt #5 received HSCT in CR1 despite mCR because of secondary AML.
AQ1 [b]Allelic burden not available.

The biological explanation of the poor outcome of this subset of patients bearing BPDCN like aberrant phenotype is unknown and might be clarified by gene expression or NGS analyses.

The infrequent aberrant coexpression of CD4, CD56, and CD123 among AML patients without *NPM1* mutations, and their heterogeneous prognosis linked to cytogenetic and molecular aberrations, prevented us to disclose any prognostic influence of IF on outcome in our *NPM1* wild-type AML cohort.

If our clinical observation are confirmed on larger series of patients, the identification of a BPDCN-like IF in NPM-mut patients may identify a subset not sharing the good prognostic impact of *NPM1* mutation. A close monitoring of MRD-clearance should be performed and patients with unsatisfactory response to therapy should be promptly addressed to more intensive strategies (eg, allogeneic stem cell transplantation in first CR). Future possibilities might be opened by the development of specific therapeutic strategies, such as monoclonal antibodies targeting CD123.

CONFLICT OF INTEREST

All authors declare that they have no conflict of interest to disclose.

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