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The Susceptibility of AML with NPM1 Mutation for Immunotherapy

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Introduction

Acute myeloid leukemia (AML) with mutated nucleophosmin 1 (NPM1mut) forms a particular entity in the WHO classification of AML (WHO classification 2017). Overall survival in patients with AML with NPM1mut is more favorable, possibly based on the immunogenicity of the mutated NPM1. Thus, one can hypothesize that AML with NPM1mut might constitute an immunogenic subtype of AML.

In a survival analyses of 25 NPM1mut AML we found that patients that appeared to have specific T-cell responses against one or two of the immunogenic peptides had a better overall survival in comparison to those cases showing no specific immune responses [1]. These findings show that immune responses against the mutated protein of *NPM1* might contribute to the favourable prognosis of AML patients carrying *NPM1mut*. Clinical data also suggest that AML with *NPM1mut* correlates in general with better prognosis [2]; however, the underlying mechanisms still need to be further investigated.

In 2012, our group described specific T cell responses against epitopes derived of the mutated region of Nucleophosmin 1 (*NPM1mut*) [3]. With the help of bioinformatics, amino acid sequences of wild-type and mutated NPM1 were screened as to their predictive scores via the programs SYFPEITHI, Rankpep and BIMAS. Out of ten 9-mer peptides, two HLA-A2 restricted NPM1-mutated peptides (AIQDLCLAV and AIQDLCVAV) were found to be immunogenic in *NPM1mut* AML patients. Additionally, Kuzelova et al. compared the frequency of human leukocyte antigen allele I (HLA-I) in AML with NPM1mut with AML with NPM1wt [4]. They observed that in AML with NPM1mut several HLA-I alleles were associated with better overall survival. They concluded that these patients in a predisposition to develop an efficient anti-AML immune response against the cytoplasmatic located and immunogenic accessible mutated NPM1. Our group investigated in microarray analysis whether the enriched leukemic stem cell (LSC) population of AML with NPM1mut shows an expression profile that may make AML with NPM1mut susceptible for immune mechanisms [5]. CD96 and IL12RB1 are interesting target structures as they were shown to be significantly higher expressed in the LSC fraction of AML with NPM1mut compared to NPM1wt and underline the potential relevance of immune mechanisms in AML with NPM1mut [5]. CD96 belongs to the immunoglobulin superfamily and has a role in adhesive interactions of activated T and NK cells and it has functions in antigen presentation [6]. It also seems to be a further checkpoint inhibitor with potential clinical relevance for tumor control [7]. Interleukin-12 (IL-12) in general is thought to have a potent antitumor activity through immune stimulatory and anti angiogenic mechanisms and has direct activity on tumor cells. Moreover, IL-12 might directly inhibit the leukemic cell growth and therefore the IL-12/IL12RB1 interaction might be of remarkable relevance for leukemic cell rejection [8].

Currently, immune checkpoint inhibitors which target PD1 and PD-L1 are applied successfully in patients with solid tumors as well as Hodgkin lymphoma. In order to learn about PD-L1 expression in AML with NPM1mut, we screened 30 AML patients (15 NPM1mut AML and 15 NPM1wt AML patients). Expression of CD34/CD38/CD274 (PD-L1) was tested via flowcytometry. We observed that many AML cases had relevant expression of PD-L1 (CD274) and that bulk AML cells (all cells except CD34+CD38- cells) of *NPM1mut* AML showed a significantly higher PD-L1 expression in comparison to *NPM1wt* AML patients (median of 1.4% positive cells versus 0.25%; $p<0.0001$; Figure 1). Remarkably, in leukemic progenitor/stem cells (CD34+CD38-) PD-L1 expression was detected at a higher percentage in NPM1mut than in NPM1wt AML (median of 3.6% positive cells versus 0.29%; $p<0.0001$; Figure 1) [9]. In FACS analysis data of representative patient samples a similar pattern can be observed. Figure 2 shows data of a NPM1mut patient, where a smaller LSC fraction is seen, however of these cells a clear CD274+ fraction of 4.27% could be detected. In turn, in Figure 3, the NPM1wt patient had a larger LSC fraction; however

the CD274+ fraction detected was much smaller. Figure 4 shows the expression pattern of all 30 patients, divided into NPM1mut and NPM1wt patients.

Flow cytometry analysis of CD274 expression in AML (Figures 1-4).

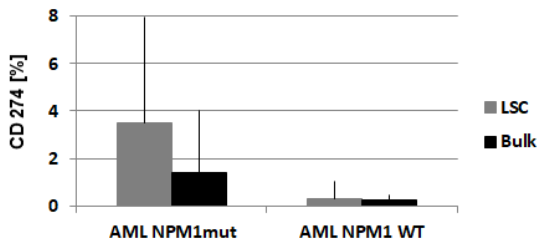

Figure 1. More LSC compartment cells of *NPM1mut* AML patients showed PD-L1 expression compared to *NPM1wt* cases, and likewise more Bulk cells of *NPM1mut* AML patients showed PD-L1 expression compared to *NPM1wt* cases (in both cases, p<0.0001).

Typical examples of FACS analysis of PD-L1 expression in LSC-enriched cells of one *NPM1mut* patient and one *NPM1wt* AML patient.

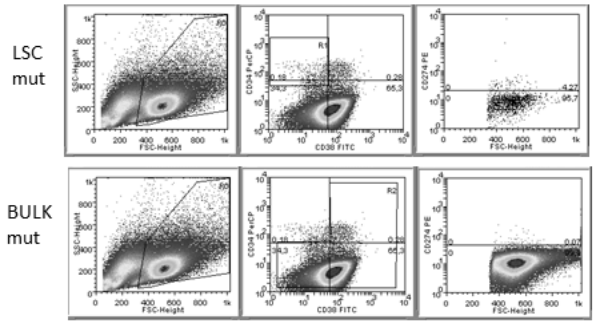

Figure 2. A *NPM1mut* AML patient with a relatively high percentage of 4.27% PD-L1 expressing cells in the LSC fraction.

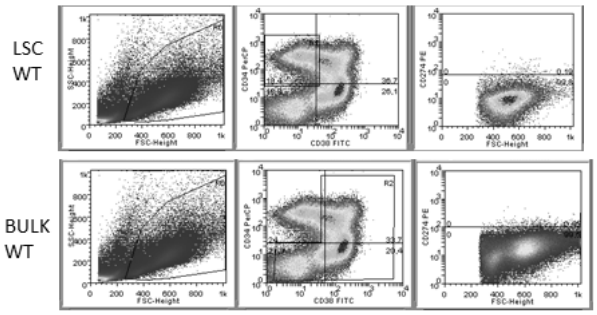

Figure 3. Shows an exemplary *NPM1wt* AML patient with low PD-L1 expression.

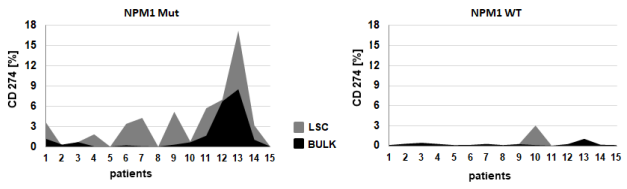

Figure 4. All 30 Patients displayed, with a significantly higher PD-L1 expression in the *NPM1mut* AML patients.

Our findings underline that *NPM1mut* patients might be better candidates for immune checkpoint PD-1/PD-L1-driven immunogenic approaches than AML subtypes without NPM1mut. Immune checkpoint inhibition following chemotherapy as a maintenance therapy might be an effective tool to prevent relapse. Especially, as the immune response against leukemic cells is stronger in *NPM1mut* compared to *NPM1wt* AML patients [1]. Immune responses that are possibly responsible for the high cure rate in *NPM1mut* AML cohort, might further be improved via PD-1/PD-L1 inhibition in this immunogenic subgroup of AML.

In conclusion, AML with NPM1mut represents an immunological subtype, as specific T-cell responses are detected against NPM1mut specific peptides and a higher PD-L1 expression was shown in the leukemic progenitor/stem cell compartment compared to NPM1wt AML. Additionally, in a retrospective survival analyses patients with specific T-cell responses against immunogenic peptides derived from NPM1mut had a better overall survival in comparison to the cases showing no specific immune responses. Therefore, the hypothesis that the prognostic favorability in AML with NPM1mut is based on immunological effects is underlined. The immunogenicity of the neoantigen NPM1mut and the higher PD-L1 expression are promising target structures for individualized immunotherapeutic approaches. PD-1/PD-L1-directed immune checkpoint inhibition to enhance *NPM1mut* specific T cell responses might be combined with antigen-directed immunotherapies e.g. peptide vaccination against immunogenic NPM1 epitopes in order to eradicate persisting MRD following conventional chemotherapy.

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Conflict of Interest

JG received funds from BMS (Bristol-Myers Squibb); the other authors do have no potential conflicts of interest with regard to this study.

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