ORIGINAL ARTICLE

Title

CIRCULATING INNATE IMMUNE MARKERS AND OUTCOMES IN TREATMENT-NAÏVE ADVANCED NON-SMALL CELL LUNG CANCER (NSCLC) PATIENTS

Authors:

M. Charrier^{1,2,3*}, L. Mezquita^{1,2,4*}, B. Lueza^{5,6}, L. Dupraz^{1,2}, D. Planchard^{1,4}, J. Remon^{1,4}, C. Caramella⁷, L. Cassard^{1,2}, L. Boselli^{1,2}, K. S. Reiners⁸, E. Pogge von Strandmann⁹, S. Rusakiewicz^{1,10}, R. Ferrara^{1,2}, B. Duchemann^{1,2,3}, M. Naigeon^{1,2,3}, J.P. Pignon^{5,6}, B. Besse^{1,3,4#}, N. Chaput^{1,2,11#}

Affiliations:

¹ Gustave Roussy Cancer Campus, F-94805, Villejuif, France;

² Laboratory of Immunomonitoring in Oncology, UMS 3655 CNRS/US 23 INSERM, Gustave Roussy Cancer Campus, F-94805, Villejuif, France;

³ University Paris-Saclay, Faculty of Medicine, F-94270, Le Kremlin-Bicêtre, France;

⁴ Department of Cancer Medicine, Gustave Roussy Cancer Campus, F-94805, Villejuif, France;

⁵ Biostatistics and Epidemiology Department, University Paris Saclay, Gustave Roussy Cancer Campus, F-94805, Villejuif, France;

⁶ Oncostat CESP, INSERM, University Paris-Saclay; UVSQ, University Paris-Sud, F-94085, Villejuif, France

⁷ Radiology Department, Gustave Roussy, Cancer Campus, F-94085, Villejuif, France

⁸ Institute for Clinical Chemistry and Clinical Pharmacology, University Hospital Bonn, 53127 Bonn, Germany

⁹ Experimental Tumor Research, Center for Tumor Biology and Immunology, Clinic for Hematology, Oncology and Immunology, Philipps University, 35043 Marburg, Germany

¹⁰ Center of Clinical Investigations in Biotherapies of Cancer (CICBT) 1428, Villejuif, France

¹¹ University Paris-Saclay, Faculty of Pharmacy, Chatenay-Malabry, F-92296, France.

* MC and LM contributed equally to this work

NC and BB contributed equally to this work

CORRESPONDING AUTHOR:

Professor Nathalie Chaput

Laboratoire d'Immunomonitoring en Oncologie

Gustave Roussy Cancer Campus

114 rue Edouard Vaillant

94805 Villejuif, France

+33 1 42 11 56 55

Email: nathalie.chaput@gustaveroussy.fr

Running title: NCR3 is an independent prognostic factor in advanced NSCLC

Conflict of interests: The authors declare no potential conflicts of interest

Figures and tables (Maximum 6 figures and tables): 4 figures, 2 tables

References: 32

Supplementary, figures and tables: 1 figures, 7 tables

KEY WORDS: NSCLC, innate cells, Natural Cytotoxicity Receptor, NCR3/NKp30.

Funding: This study was funded by Gustave Roussy Cancer Campus, Fondation Gustave Roussy, the Institut national de la santé et de la recherche médicale (INSERM), SIRIC SOCRATE (INCa DGOS INSERM 6043), SIRIC SOCRATE 2.0 (INCa-DGOS-INSERM_12551), MMO program: ANR-10IBHU-0001). Dr. Laura Mezquita was recipient of the grant for DUERTECC/EURONCO 2015-2016. **Author contributions:** NC and BB were involved in the study concept and design, analysis and interpretation of data, drafting of the manuscript, obtain funding and supervision of study. MC and LM were involved in acquisition analysis and interpretation of immunological data and drafting of the manuscript. LD, DP, JRM, LC, LB, RF, BD and MN were involved in the acquisition of clinical or immunological data. KR and EPvS were involved in acquisition analysis and interpretation of BAG-6 dosage. SR was involved in acquisition analysis and interpretation of NKp30 isoforms. BL and JPP were involved in collection, analysis and interpretation of survival data, and in drafting of the manuscript. **Competing interests:** Authors declare no conflict of interests.

ABSTRACT (250 words)

Introduction. Innate immunity represents the first step of activation of the immune system and dictates the quality of adaptive immune responses. Studies have reported links between systemic inflammatory or innate immune markers and prognosis in patients with lung cancer. To our knowledge, the prospective and concomitant study of these systemic markers has been never performed. **Methods.** Advanced treatment-naïve NSCLC patients eligible to first-line of platinum-based chemotherapy were prospectively included from December 2012 to July 2015 ($N=148$). Blood sample of patient was collected before first cycle for fresh NK cell phenotyping. Peripheral blood mononuclear cells were cryopreserved for Natural Cytotoxicity Receptors (NCR) genotyping as well as sera for NCR's ligands quantification. Leukocytes, neutrophils, and monocytes counts and Lactate Dehydrogenase (LDH) levels were extracted from electronic medical records. **Results.** Among all studied markers, monocytosis, neutrophilia, leukocytosis, high LDH and sBAG6 levels and reduced levels of NCR3 transcripts were associated with poor overall survival (OS) in univariate analysis. The levels of NCR3 transcripts was linked to age, number of metastatic sites, monocyte counts, LDH and sBAG6 levels. Neutrophilia was associated to high sBAG6 levels. NCR3 was the unique innate immune parameter that remained as an independent factor associated with both OS ($P=0.003$) and progression-free survival (PFS) ($P=0.009$) in the multivariate analysis. **Conclusion.** This study brought evidence that these biomarkers are entangled; parameters associated with an inflammatory process were related to reduce levels of NCR3 transcripts. Finally, the level of NCR3 transcripts was independently associated with outcomes in treatment-naïve patients with advanced NSCLC

INTRODUCTION

The innate immune system is considered the non-specific immune system in humans, representing the immediate response to defend the host against external agents, as several pathogens, or cancer but not providing long-lasting immunity to the host (1). However, without proper activation of innate immunity the adaptive response will not be effective demonstrating the critical link between innate immunity and long-lasting protection.

In cancer patients, inflammatory status at diagnosis can negatively affect prognosis of patient and alter response to treatment (2). Recently it was shown in advanced-NSCLC patients treated with immune checkpoints blockers (ICB) that neutrophilia as well as high LDH levels could predict resistance to anti-PD-1 treatments suggesting a link between inflammatory status and the capacity to activate or reactivate properly T cells during ICB therapy (3). NK cells are the effector cells of the innate immune system, named after their ability to kill spontaneously transformed or infected cells, without prior immunization (4). Globally, NK cell cytotoxicity is determined by a balance between signaling of activating and inhibitory receptors, where the expression of Major Histocompatibility Complex-I (MHC-I) molecules and several NK receptors and ligands give them the ability to recognize target cells. The main activating receptors are the Natural killer group 2 member D (NKG2D) and the Natural Cytotoxicity triggering Receptors (NCR) commonly named NKp46/NCR1, NKp44/NCR2 and NKp30/NCR3. Previous evidence supports NK cell involvement in cancer development (5). The presence of tumor-infiltrating NK cells have been often associated with an improved disease-free survival (DFS) and better OS in several cancer types (6–8). In addition, both tumor-infiltrating and peripheral blood-NK cells of cancer patients are endowed with decreased receptor expression and unsuitable cytotoxic function (9,10). In NSCLC patients, tumor-infiltrating NK cells has been proposed as prognostic factors in early stage of NSCLC (8,11–13), showing that an efficient NK effector function is associated with better outcomes, but there is so far no solid evidence regarding the role of NK cells or other innate immune cells in advanced NSCLC disease. Several immune cells related mainly to the adaptive response, are being assessed as potential prognostic factors in advanced NSCLC in immunomonitoring studies, but so far no prior studies have evaluated the role of systemic innate immune markers in treatment-naïve advanced NSCLC population. Moreover and to our knowledge, the concomitant study of these systemic markers was never performed. In this report we studied the impact of NK cell phenotyping, levels of NCRs transcripts, NCR3 isoforms, leukocytes, neutrophils, and monocytes counts as well as levels of LDH and soluble

BAG6 on patient's outcomes in a homogeneous prospective cohort of treatment-naïve advanced NSCLC patients.

Materials and Methods

Patients and Healthy volunteers. Advanced treatment-naïve NSCLC patients (stage IIIB/IV) eligible to first-line of platinum-based chemotherapy were prospectively included from December 2012 to July 2015 in our institution ($N=148$). All patients were included in the MSN study (NCT02105168) and signed an inform consent. A peripheral blood sample (5-15 ml) of each patient was collected before the first cycle of chemotherapy. Clinical, pathological and molecular features were collected. White blood cells (WBC) count, LDH and albumin levels before treatment (from 2 days to the day of chemotherapy beginning) were also collected. Blood samples ($N=48$) of healthy volunteers (HV) were obtained after inform consent signature and were collected according to a procedure validated by the CCPSL UNT-N°12/EFS/079.

Statistical analyses. For immune parameters, statistical analyses were performed using Prism 6 software (GraphPad, San Diego, CA). P values < 0.05 were considered significant. Groups were compared using Mann-Whitney's test or Wilcoxon matched pairs test where appropriate. For correlation statistics, the Spearman's correlation test was used. OS was defined as the time from the first date of platinum-based chemotherapy use until death. Patients alive were censored at their date of last follow-up. PFS was defined as the time from the first date of platinum-based chemotherapy use to first progression or death. Patients alive without progression were censored at their date of last follow-up. OS and PFS were estimated using Kaplan-Meier method. The median follow-up was estimated using the reverse Kaplan-Meier method (14). As a first step, univariate analyses were performed with log-rank tests and univariate Cox models. Then, as a second step, multivariate analyses were performed with multivariate Cox models. Hazard ratios (HR) and their 95% Confidence Intervals (95%CI) were estimated through the Cox models to compare the risk of death or progression between

two categories. Statistical analyses were carried out using SAS version 9.3 (SAS Institute, Cary, NC).

Results

Patients' characteristics and survival

Characteristics of the 148 eligible patients are shown in Supplementary Table S1. The median duration of follow-up was 24.2 months [95%CI: 18.6; 32.4]. Patients were predominantly male (66%) with median age of 62 years [30–83 years]. Eighty-nine percent of the patients presented a stage IV and 74% had adenocarcinoma histology. All patients were previously untreated. The median OS and PFS for platinum-based chemotherapy were respectively 15.0 months [95%CI: 12.2; 16.2] and 5.9 months [95%CI: 5.0; 6.7]. All patients received platinum-based chemotherapy, with standard antiemetic treatment, including intravenous and oral steroids.

Systemic NK cell-related markers in treatment-naïve advanced NSCLC patients

Extensive fresh whole blood phenotyping of NK cells (see supplementary for methods) was realized in 70 advanced NSCLC patients and compared to 48 sex and age-matched HV. The absolute number of NK cells was similar between HV and patients (Figure 1A). The frequency of CD56^{dim} subset was increased in patients ($P=0.0016$) (Figure 1B). Since CD56^{dim} NK cells also express the CD16 activating receptor, it not surprising to observe an increase of the proportion of CD16 among NK cells in patients compared to HV (Figure 1C). Percentages of NKp44⁺ and NKp46⁺ NK cells were higher in patients compared to HV (Figure 1C); the proportion of patients, with more than 70% NKp30⁺ NK cells, was also increased in patients as compared to HV (Figure 1D). On the contrary, the surface NKG2D expression was similar to HV (Figure 1E). These different patterns of expression suggested a selective modified NCRs expression, but not for NKG2D, in advanced NSCLC patients. Since increased-expression of NCR has been assigned to NK cell activation, we monitored activation-associated molecules (HLA-DR and CD69). We did not evidence of any increase of HLA-DR (Figure 1E) or CD69 (not shown) expressions compared to HV. These results therefore suggest that peripheral NK cells in advanced NSCLC patients do have increased NCR expression, which might not be associated with cell activation.

Many reports have failed to correlate NCR transcript's level and NCR's protein level (15) (16), thus we next monitored the expression levels of NCR transcripts (see supplementary for methods). NCR3, NCR2 and NCR1 transcripts were diminished in patients compared to HV (Figure 2). These transcripts levels were highly correlated (Supplementary Figure S1) as

already described in previous works (17,18). Thus, the higher level of NCRs protein expression did not reflect the level of NCR's transcripts in these patients. Several recent studies correlated the NKp30 isoform expression pattern with the prognosis and evolution in different malignancies (16,15,19). The relative expression of each NCR3-encoded NKp30 isoforms (NKp30A, NKp30B and NKp30C) was significantly lower in patients than in HV (Supplementary Figure S1), in line with a global diminution of NCR3 transcripts; note that transcripts levels of each NKp30 isoforms were highly correlated to NCR3 transcripts levels (Supplementary Figure S1). We assessed the relative expression of NKp30 isoforms compared with each other, using the "delta" formula as described elsewhere (15). No difference between ΔAB , ΔAC and ΔBC was observed in patients with late stage NSCLC compared to HV (Supplementary Figure S1), indicating a global decrease of NCR3 transcripts with a maintained hierarchy of each isoform in patients with advanced stage NSCLC. Altogether patients with advanced stage NSCLC patients have a global decrease of NCRs transcripts levels.

Systemic routine blood innate immune markers and NK cell-related markers and prognosis in treatment-naïve advanced NSCLC patients

We studied the routine blood parameters, mainly total leukocyte, neutrophil, lymphocyte, monocyte count and LDH as well as NK cells phenotype and NCRs transcripts. Univariate analyses were performed first and showed that monocytosis ($P=0.011$), neutrophilia ($P<0.001$), high LDH levels ($P=0.008$), derived neutrophil to lymphocyte ratio (dNLR) ($P=0.033$) were associated to OS (Supplementary Table S2) while only neutrophilia ($P=0.022$) remained associated to PFS in these patients that received platinum-based chemotherapy as first line treatment (Supplementary Table S3).

NCR3 transcripts and its three isoforms A/B/C, NCR2, NCR1 and percentNKp30 have been categorized based on the tertiles (T1=33.3% and T2=66.6%) of their distribution. Only NCR3 tertiles were significantly associated with OS (trend test $P=0.019$) and a trend for PFS (trend test $P=0.057$). Other biomarkers related to NK cells were not associated with OS in univariate analyses (Figure 3).

NCR3 relative expression according to other clinical or systemic innate immune parameters

Age at inclusion (Chi² test, $P=0.047$) and the number of metastatic sites (Chi² test, $P=0.022$) were significantly associated with levels of NCR3 transcripts (Supplementary Table S4). Monocytosis was strongly linked to levels of NCR3 transcripts (Chi² test, $P=0.005$) and to lower LDH levels (Chi² test, $P=0.033$) (Supplementary Table S5). Endogenous soluble forms

of NKp30 ligands known to date are sBAG6 and sB7-H6. These soluble ligands were described to engage the NKp30 receptor and dampen its activity when shed in the serum or in supernatants (15,20,21). No significant levels of sB7-H6 were found in these patients. For sBAG6 (see supplementary for methods), a large majority of patients have detectable serum levels of sBAG6 (>0.5 ng/ml) and 36% presented with important levels of sBAG6 (>5 ng/ml). Levels sBAG6 were associated with low levels of NCR3-transcripts (Figure 4) and neutrophilia (Chi² test, $P=0.016$) (Supplementary Table S6).

The Level of NCR3 transcripts and neutrophils are independently associated with outcomes in patients with treatment-naïve advanced NSCLC.

Among blood measures, which were significantly associated with OS or PFS, monocytosis, neutrophilia, LDH and level of NCR3 transcripts were assessed in the multivariate Cox model. As neutrophilia and leukocytosis were highly correlated, neutrophilia was chosen above leukocytosis. For sBAG6 even significantly related to OS in univariate analysis, the small number of patients with sBAG6 quantification ($N=65$) led us to skip this systemic biomarker. After adjustment in the final Cox model, NCR3 transcripts (trend test, $P=0.003$; Table 1) and Neutrophilia ($P=0.028$; Table 1) remained associated with OS and NCR3 transcripts remained the unique innate immune marker associated with PFS (trend test, $P=0.009$; Table 2).

At time of the cut-off data (December 2016), only 15 patients received anti-PD1 as 2nd line or beyond (10%). Among them, only 4 events for OS (death) were registered. Considering the small sample size, No statistical analysis were done.

DISCUSSION

In our work, we demonstrated that the innate immune system, particularly NK cell-related markers, can have a prognostic impact in treatment-naïve advanced NSCLC. Neutrophils and NCR3 transcript levels were independent prognostic factors in this population. To our knowledge, this is the largest study that has specifically assessed the prognostic impact of systemic immune innate biomarkers simultaneously in a homogeneous and prospective cohort of treatment-naïve advanced NSCLC patients.

In our cohort, we did not detect any difference in absolute number of NK cells compared to sex and age matched healthy donors. Considering NK cell phenotype, an increased frequency of CD56^{dim} subset while no difference could be revealed for NKG2D as already described in very old healthy donors (> 80 years) (22). Increased cell-surface expression of NCRs proteins with global decrease of NCRs transcripts may seem contradictory. However, NKp30 receptor

is associated with CD3 ζ and Fc γ RIII(23), being necessary for its cell surface expression. Thus a discrepancy between the level of NCR3 transcripts and NKp30 surface expression can be observed, as already described (24). In our cohort we observed a coordinated diminution of NCRs but only the level of NCR3 transcripts has been associated with prognosis. Cell surface NKp30 expression on NK cells was not related to outcome. This seems consistent with previous observations demonstrating that diminution of NCRs transcripts can be associated with the decrease of cytotoxic NK cell functions while no association between protein expression and NCR3 transcript levels could be detected (19,25). In our cohort, we assessed NK cell effector functions on ex-vivo purified NK cells from 8 advanced NSCLC patients. Granzyme B and perforin secretions after NKp30 engagement correlated with levels of NCR3 transcripts (data not shown). Thus the low level of NCR3-transcripts was associated with diminished NK cell cytotoxic functions. Coordinated diminution of NCR1, NCR2 and NCR3 has been described by others (18) in human, ETS-1 transcription factor has been shown to bind to an *in silico* predicted region of NCR promoters. ETS-1 gene expression was decreased in presence of tumor cells and this was associated with the diminution of NCR1, NCR2 and NCR3.

As in melanoma (16), no differences between NKp30 isoforms from patients and HV was observed suggesting that balance between isoforms might not play a significant role in both populations. This data differs from Fend *et al* findings, where low level of NCR3 transcripts and a switch on the relative proportion of NKp30 isoforms was observed compared to HV (26). At that time, they compared advanced NSCLC with young HV (median age 38 years). Since the relative proportion of NKp30 isoforms is altered in elderly, a more reliable comparison with sex and age-matched HV is required. In our study comparing patients with sex and age-matched HV led us to conclude that the relative expression of NKp30 isoforms was maintained in our cohort.

Systemic inflammatory status can impact on the function of innate/adaptive immune responses (2) and has been correlated with worse prognosis in lung cancer, mainly in early stage NSCLC (27,28). Neutrophil, monocyte counts and LDH levels were associated with the level of NCR3 transcripts or concentration of sBAG6 in the serum of patients. Neutrophils, but not LDH and monocytes, remained independently associated with OS in our population. The presence of ≥ 7.5 G/L neutrophils before treatment was an independent prognostic factor, similarly to prior studies, and may reflect the pro-inflammatory status in cancer. Interestingly, sBAG6 was correlated with the absolute number of neutrophils. These observations led us to

the conclusion that increased concentration of sBAG6 and diminished levels of NCR3 transcripts could be the result of cancer-related inflammation.

Low levels of NCR3 transcripts were related to higher number of metastatic sites, reminiscent of the already published concept of an anti-metastatic role of NK cells (29,30). Secretion of ligands such as sBAG6 that can decrease NCR3 transcripts could disrupt NK cell functions and might represent an escape mechanism linked to metastatic spread in these patients. Altogether NK cells and particularly NCR3 could represent good targets in patients with advanced NSCLC. NK cells have the ability to modulate the adaptive immune system through cytokine production or direct cell to cell interactions (31,32). An alteration of NK functions could be a mechanism associated with impairment of adaptive immunity in these patients with cancer. We recently suggested that neutrophils and LDH may predict response to ICB (3). More recently a study demonstrated that among patients who achieved clinical responses after treatment with nivolumab a significantly higher baseline transcriptional signature of NK cells, $\gamma\delta$ T cells and interleukin-15 was observed compared with patients with progressive disease (33). Altogether, these data suggest that innate immunity might represent an important counterpart to reach a clinical response during anti-PD-1 / PD-L1 treatments. The small size of this population treated subsequently with anti-PD1 (N=19) did not allow drawing solid conclusions and larger studies should evaluate the role of NK in patients treated with anti-PD-1 / PD-L1 treatments. Thereby, we propose that dampening cancer-related inflammation to restore NK cell functions might represent a good therapeutic strategy to boost efficacy of immunotherapy.

CONCLUSION (92 words)

Prospective and concomitant evaluation of several systemic innate markers in never treated patients with advanced NSCLC brought evidence that parameters usually associated with an inflammatory process as monocytosis, neutrophilia, important levels of sBAG6 and of LDH were related to reduce levels of NCR3 transcripts. Only diminished levels of NCR3 remained independently associated with both PFS and OS. Recent studies suggested that innate immunity might be associated to response to immune checkpoints blockers targeting PD-1/PD-L1. Thus, the predictive value of these systemic innate/inflammatory markers before the introduction of anti-PD-1/PD-L1 should be realized prospectively.

Bibliography (33 ref)

1. Hato T, Dagher PC. How the Innate Immune System Senses Trouble and Causes Trouble. *Clin J Am Soc Nephrol CJASN*. 2015;10:1459–69.
2. Botta C, Misso G, Martino EC, Pirtoli L, Cusi MG, Tassone P, et al. The route to solve the interplay between inflammation, angiogenesis and anti-cancer immune response. *Cell Death Dis*. 2016;7:e2299.
3. Mezquita L, Auclin E, Ferrara R, Charrier M, Remon J, Planchard D, et al. Association of the Lung Immune Prognostic Index With Immune Checkpoint Inhibitor Outcomes in Patients With Advanced Non-Small Cell Lung Cancer. *JAMA Oncol*. 2018;4:351–7.
4. Morvan MG, Lanier LL. NK cells and cancer: you can teach innate cells new tricks. *Nat Rev Cancer*. 2016;16:7–19.
5. Imai K, Matsuyama S, Miyake S, Suga K, Nakachi K. Natural cytotoxic activity of peripheral-blood lymphocytes and cancer incidence: an 11-year follow-up study of a general population. *Lancet Lond Engl*. 2000;356:1795–9.
6. Coca S, Perez-Piqueras J, Martinez D, Colmenarejo A, Saez MA, Vallejo C, et al. The prognostic significance of intratumoral natural killer cells in patients with colorectal carcinoma. *Cancer*. 1997;79:2320–8.
7. Rusakiewicz S, Semeraro M, Sarabi M, Desbois M, Locher C, Mendez R, et al. Immune infiltrates are prognostic factors in localized gastrointestinal stromal tumors. *Cancer Res*. 2013;73:3499–510.
8. Villegas FR, Coca S, Villarrubia VG, Jiménez R, Chillón MJ, Jareño J, et al. Prognostic significance of tumor infiltrating natural killer cells subset CD57 in patients with squamous cell lung cancer. *Lung Cancer Amst Neth*. 2002;35:23–8.
9. Costello RT, Sivori S, Marcenaro E, Lafage-Pochitaloff M, Mozziconacci M-J, Reviron D, et al. Defective expression and function of natural killer cell-triggering receptors in patients with acute myeloid leukemia. *Blood*. 2002;99:3661–7.
10. Le Maux Chansac B, Moretta A, Vergnon I, Opolon P, Lécluse Y, Grunenwald D, et al. NK cells infiltrating a MHC class I-deficient lung adenocarcinoma display impaired cytotoxic activity toward autologous tumor cells associated with altered NK cell-triggering receptors. *J Immunol Baltim Md 1950*. 2005;175:5790–8.
11. Bruno A, Focaccetti C, Pagani A, Imperatori AS, Spagnoletti M, Rotolo N, et al. The proangiogenic phenotype of natural killer cells in patients with non-small cell lung cancer. *Neoplasia N Y N*. 2013;15:133–42.
12. Carrega P, Morandi B, Costa R, Frumento G, Forte G, Altavilla G, et al. Natural killer cells infiltrating human nonsmall-cell lung cancer are enriched in CD56 bright CD16(-) cells and display an impaired capability to kill tumor cells. *Cancer*. 2008;112:863–75.
13. Platonova S, Cherfils-Vicini J, Damotte D, Crozet L, Vieillard V, Validire P, et al. Profound coordinated alterations of intratumoral NK cell phenotype and function in lung carcinoma. *Cancer Res*. 2011;71:5412–22.

- 381 14. Schemper M, Smith TL. A note on quantifying follow-up in studies of failure time.
382 Control Clin Trials. 1996;17:343–6.
- 383 15. Messaoudene M, Fregni G, Enot D, Jacquelot N, Neves E, Germaud N, et al. NKp30
384 isoforms and NKp46 transcripts in metastatic melanoma patients: unique NKp30 pattern
385 in rare melanoma patients with favorable evolution. OncoImmunology. 2016;0:00–00.
- 386 16. Semeraro M, Rusakiewicz S, Minard-Colin V, Delahaye NF, Enot D, Vély F, et al.
387 Clinical impact of the NKp30/B7-H6 axis in high-risk neuroblastoma patients. Sci
388 Transl Med. 2015;7:283ra55.
- 389 17. Ramirez K, Chandler KJ, Spaulding C, Zandi S, Sigvardsson M, Graves BJ, et al. Gene
390 Deregulation and Chronic Activation in Natural Killer Cells Deficient in the
391 Transcription Factor ETS1. Immunity. 2012;36:921–32.
- 392 18. Venton G, Labiad Y, Colle J, Fino A, Afridi S, Torres M, et al. Natural killer cells in
393 acute myeloid leukemia patients: from phenotype to transcriptomic analysis. Immunol
394 Res. 2016;64:1225–36.
- 395 19. Delahaye NF, Rusakiewicz S, Martins I, Ménard C, Roux S, Lyonnet L, et al.
396 Alternatively spliced NKp30 isoforms affect the prognosis of gastrointestinal stromal
397 tumors. Nat Med. 2011;17:700–7.
- 398 20. Pogge von Strandmann E, Simhadri VR, von Tresckow B, Sasse S, Reiners KS, Hansen
399 HP, et al. Human leukocyte antigen-B-associated transcript 3 is released from tumor
400 cells and engages the NKp30 receptor on natural killer cells. Immunity. 2007;27:965–74.
- 401 21. Matta J, Baratin M, Chiche L, Forel J-M, Cognet C, Thomas G, et al. Induction of B7-
402 H6, a ligand for the natural killer cell-activating receptor NKp30, in inflammatory
403 conditions. Blood. 2013;122:394–404.
- 404 22. Hazeldine J, Lord JM. The impact of ageing on natural killer cell function and potential
405 consequences for health in older adults. Ageing Res Rev. 2013;12:1069–78.
- 406 23. Pende D, Parolini S, Pessino A, Sivori S, Augugliaro R, Morelli L, et al. Identification
407 and molecular characterization of NKp30, a novel triggering receptor involved in natural
408 cytotoxicity mediated by human natural killer cells. J Exp Med. 1999;190:1505–16.
- 409 24. Zhang T, Wu M-R, Sentman CL. An NKp30-based chimeric antigen receptor promotes
410 T cell effector functions and antitumor efficacy in vivo. J Immunol Baltim Md 1950.
411 2012;189:2290–9.
- 412 25. Ménard C, Blay J-Y, Borg C, Michiels S, Ghiringhelli F, Robert C, et al. Natural killer
413 cell IFN-gamma levels predict long-term survival with imatinib mesylate therapy in
414 gastrointestinal stromal tumor-bearing patients. Cancer Res. 2009;69:3563–9.
- 415 26. Fend L, Rusakiewicz S, Adam J, Bastien B, Caignard A, Messaoudene M, et al.
416 Prognostic impact of the expression of NCR1 and NCR3 NK cell receptors and PD-L1
417 on advanced non-small cell lung cancer. Oncoimmunology. 2017;6:e1163456.

27. Hu P, Shen H, Wang G, Zhang P, Liu Q, Du J. Prognostic significance of systemic inflammation-based lymphocyte- monocyte ratio in patients with lung cancer: based on a large cohort study. *PloS One*. 2014;9:e108062.
28. Paramanathan A, Saxena A, Morris DL. A systematic review and meta-analysis on the impact of pre-operative neutrophil lymphocyte ratio on long term outcomes after curative intent resection of solid tumours. *Surg Oncol*. 2014;23:31–9.
29. Desbois M, Rusakiewicz S, Locher C, Zitvogel L, Chaput N. Natural killer cells in non-hematopoietic malignancies. *Front Immunol*. 2012;3:395.
30. Yin T, Wang G, He S, Liu Q, Sun J, Wang Y. Human cancer cells with stem cell-like phenotype exhibit enhanced sensitivity to the cytotoxicity of IL-2 and IL-15 activated natural killer cells. *Cell Immunol*. 2016;300:41–5.
31. Gerosa F, Baldani-Guerra B, Nisii C, Marchesini V, Carra G, Trinchieri G. Reciprocal activating interaction between natural killer cells and dendritic cells. *J Exp Med*. 2002;195:327–33.
32. Martín-Fontecha A, Thomsen LL, Brett S, Gerard C, Lipp M, Lanzavecchia A, et al. Induced recruitment of NK cells to lymph nodes provides IFN-gamma for T(H)1 priming. *Nat Immunol*. 2004;5:1260–5.
33. Rodig S.J, Gusenleitner D, Jackson D.G, Gjini E, Giobbie-Hurder A, Jin C, et al. MHC proteins confer differential sensitivity to CTLA-4 and PD-1 blockade in untreated metastatic melanoma. *Sci Transl Med*. 2018;10(450).

Figure 1. Phenotype of circulating NK cells from donors and advanced NSCLC. (A) Absolute numbers and (B) proportion of circulating NK cells, CD56^{bright} and CD56^{dim} cells. (C) Levels of NK receptors (CD16, NKp30, NKp44, NKp46) and (D) proportion of patients expressing high (>70%), normal (50-70%) and low levels (<50%) of NKp30 compared to healthy volunteers (HV). (E) Proportion of NKG2D and CD69 in the peripheral whole blood CD3⁺ CD56⁺ NK cells of HV and NSCLC patients. Each dot represents one patient. Groups were compared using Mann-Whitney's test. ***p*<0.01; ****p*<0.001; and *ns* means no significant.

Figure 2. Relative expression of NCR1, NCR2 and NCR3, by qRT-PCR in peripheral blood mononuclear cells (PBMC) in advanced NSCLC patients and 29 healthy volunteers (HV). Each dot represents one patient. Groups were compared using Mann-Whitney's test. $**p<0.01$; $****p<0.0001$.

A OS

B PFS

Figure 3: (A) Overall survival (OS) and (B) Progression Free Survival of treatment-naïve advanced NSCLC patients according to NCR3 transcripts.

Figure 4: Relative expression of NCR and NKp30 isoform according to sBAG6. (A) Percentage of advanced NSCLC patients and HV with high serum levels of sBAG6 (> 5ng/ml). (B) NCR3 transcripts according to sBAG6 median. Each dot represents one patient. Groups were compared using Mann-Whitney's test. *p<0.05

TABLES

Table 1. Multivariate analysis of overall survival (N=107).

Variable	HR [95% CI]	P-value	Overall P-value (trend test)
NKp30			
1	1 (ref)		
2	0.50 [0.27; 0.95]	0.034	0.008
3	0.32 [0.15; 0.66]	0.002	(0.003)
Age at inclusion			
< 60 years	1.08 [0.48; 2.47]	0.851	0.963
60-69 year	1.12 [0.50; 2.48]	0.784	
≥ 70 year	1 (ref)		
Smoking status			
Non smoker	1 (ref)		0.113
Former smoker	3.59 [1.06; 12.13]	0.039	
Current smoker	3.02 [0.84; 10.79]	0.090	
Histology			
Adenocarcinoma	0.72 [0.39; 1.36]	0.315	0.315
Non-adeno	1 (ref)		
Stage			
Stage IIIb	0.35 [0.12; 1.02]	0.053	0.053
Stage IV	1 (ref)		
Number of metastatic sites			
< 3	0.50 [0.26; 0.95]	0.033	0.033
≥ 3	1 (ref)		
Monocytosis			
< 0.8 x 10 ⁹ /L	1 (ref)		0.191
≥ 0.8	1.46 [0.83; 2.56]	0.191	
Neutrophilia			

< 7.5 x 10 ⁹ /L	1 (ref)		0.028
≥ 7.5	1.94 [1.07; 3.50]	0.028	
LDH			
< 248 U/L (ULN)	1 (ref)		0.314
≥ 248 U/L	1.41 [0.72; 2.75]	0.314	

Table 2: Multivariate analysis of progression-free survival (N=107).

Variable	HR [95% CI]	P-value	Overall P-value (trend test)
NKp30			
1	1 (ref)		0.034 (0.009)
2	0.66 [0.36; 1.21]	0.181	
3	0.43 [0.23; 0.82]	0.010	
Age at inclusion			
< 60 years	0.84 [0.42; 1.69]	0.627	0.551
60-69 year	1.11 [0.57; 2.14]	0.767	
≥ 70 year	1 (ref)		
Smoking status			
Non smoker	1 (ref)		0.016
Former smoker	3.92 [1.54; 9.96]	0.004	
Current smoker	3.60 [1.31; 9.87]	0.013	
Histology			
Adenocarcinoma	0.68 [0.39; 1.19]	0.174	0.174
Non-adeno	1 (ref)		
Stage			
Stage III	0.29 [0.13; 0.65]	0.003	0.003
Stage IV	1 (ref)		
N# of metastasic sites			
<3	0.69 [0.38; 1.25]	0.217	0.217
≥ 3	1 (ref)		

Variable	HR [95% CI]	<i>P-value</i>	<i>Overall P-value (trend test)</i>
Monocytosis			
< 0.8 x 10 ⁹ /L	1 (ref)		
≥ 0.8	1.32 [0.78; 2.23]	0.308	0.308
Neutrophilia			
< 7.5 x 10 ⁹ /L	1 (ref)		
≥ 7.5	1.06 [0.64; 1.76]	0.811	0.811
LDH			
< 248 U/L (ULN)	1 (ref)		
≥ 248 U/L	0.80 [0.45; 1.44]	0.459	0.459

486

487

488

489

490

491

LIST OF SUPPLEMENTARY MATERIAL

- **Supplementary methods.**
- **Figure S1:** Correlation of NKp30 isoform levels with other NK markers.
- **Table S1:** Patient's and healthy volunteer's characteristics.
- **Table S2.** Univariate analysis of overall survival (OS)
- **Table S3.** Univariate analysis of progression-free survival (PFS)
- **Table S4:** Clinical characteristics according to NKp30 status ($N=148$).
- **Table S5:** Blood parameters according to NKp30 status ($N=148$).
- **Table S6:** Blood parameters according to sBAG6 status ($N=65$).
- **Table S7:** qRT-PCR primers and TaqMan probes sequences.

Supplementary methods.

Flow cytometry analysis. Phenotyping analysis was performed on whole blood and Peripheral blood mononuclear cells (PBMC) that were isolated by Ficoll density gradient. Whole blood was incubated with fluorochrome-conjugated antibodies (Table S7) for 15 min at room temperature, followed by 15 min of Red Blood Cell lysis (Versalyse and Fixative solution, Beckman Coulter). Stained cells were acquired using a Canto II flow cytometer (BD) and a Gallios flow cytometer (Beckman Coulter). Data were analyzed using Kaluza software (Beckman Coulter).

Quantitative reverse transcription PCR and determination of NKp30 profiling. Total cellular RNA was isolated from PBMC or cell lines with the RNeasy kit (Qiagen). First strand cDNA was synthesized from 5 µg of total RNA using SuperScript III reverse transcriptase (Invitrogen, 18080-044) and random primers (Promega, C1181) according to Invitrogen's instructions. For the qRT-PCR, 5 µL of first-strand cDNA was mixed with 12.5 µL of 2X TaqMan Gene Expression Master Mix (Applied Biosystems) and 0.75 µL of NKp30 primers (10 µM) and probe (5 µM), 0.5 µL of β 2M primers (10 µM) and probe (5 µM) or 1 µL of NCR3, NCR2 or NCR1 sonde mixed in a final volume of 25 µL (Table S8). Temperature cycling and real time fluorescence measurement were done using a StepOnePlus System (Applied Biosystems).

The PCR conditions were as follows: initial incubation at 50 °C for 2 min, denaturation at 95 °C for 10 min, followed by 45 cycles at 95 °C for 15 s and 60 °C (for NKp30A, B, and C, NCR1,2 and 3 and β 2M transcripts) for 1 min. The PCR primers and TaqMan probes are listed in Supplementary Table 8. Analysis of relative gene expression was performed with the $2^{-\Delta C_t}$ method normalized to the level of expression of the housekeeping gene β -2-microglobulin. The formula used is $2^{-\Delta C_t} = 2^{[C_t(\text{reference gene}) - C_t(\text{sample})]} \times 10^6$. The expression of the distinct NKp30 isoforms compared with each other in each patient (delta) was calculated using the "delta" formula [Δ NKp30a NKp30b = NKp30b - NKp30a] for Δ AB,

[Δ NKp30a NKp30c = NKp30c - NKp30a] for Δ AC, [Δ NKp30b NKp30c = NKp30c - NKp30b] for Δ BC as described elsewhere (15).

Quantification of BAG6. Detection of BAG6 was performed using the sandwich ELISA procedure. Plates were coated overnight at 4°C with mouse monoclonal 3E4 (raised against the BAG6 N-terminus) as capture antibody and blocked with 1% BSA-PBS for 1h at room temperature (RT). Standard and plasma/serum were added for 1.5h at RT. Bound BAG6 was detected using the polyclonal chicken anti-BAG6 (13pp2, raised against recombinant BAG6 N-terminus) followed by goat anti-chicken POD. The assay was developed with 100µl 1-step ultra TMB ELISA solution (ThermoScientific). The reaction was stopped after 20 min with 50µl of 30% sulfuric acid, and absorbance was measured at 450nm using SpectraMax Microplate Reader (Marshall Scientific).

Evaluation of NK cell effector functions. A 96-well Maxisorp® plates were coated with anti-NKp30 mAbs (clone 210847, R&D), or the respective isotype control mAbs (each at 2.5 µg/ml in 50 µL/well of sterile PBS 1X). Plates were incubated 2 hours at 37°C. NK cells were enriched from freshly harvested blood sample with NK cells Easy Sep kit (Stem cell Technologies) and seeded at 0.5x10⁵ cells/well (RPMI 10% ABserum) in triplicate with IL-2 (100UI/mL) for 24 h at 37 °C. Supernatant were harvested and IFN γ , IL-10 (Biolegend), TNF α (BD) and VEGF (R&D) were measured by commercial ELISA kits according to manufacturer's instructions. CXCL9 (MIG), CXCL11 (I-TAC), Granzyme A, Granzyme B and Perforin were quantified by the 5-plex Human cytokine/CD8 magnetic bead panel Milliplex kit (Milipore) using Magpix (Luminex).

552 **Supplementary Figure S1**

Figure S1. Correlation of NKp30 isoform levels with other NK markers. (A) Spearman correlations between NCR relative expressions. (B) Relative expression of NKp30a, NKp30b and NKp30c in HV and NSCLC patient. (C) Spearman correlations between NKp30 isoforms and NCR3 relative expression. (D) Δ AB, Δ AC, Δ BC in HV and NSCLC patients. (E) Spearman correlations between NKp30 isoforms, NCR3 relative expression and percentage of NKp30+. (F) Spearman correlations between NKp30 isoform, NCR3 relative expression and percentage of blood NK cells.

SUPPLEMENTARY TABLES

Table S1: Patient's and healthy volunteer's characteristics.

	<i>Patients</i> <i>N=148 (%)</i>	<i>Healthy</i> <i>Volunteers</i> <i>N=48 (%)</i>
Sex		
Male	98 (66%)	31 (65%)
Female	50 (34%)	17(35%)
Age at diagnosis (years)		
Median	62	60
Range	30 ; 83	43 ; 81
Age at diagnosis (years)		
< 60	55 (37%)	
60-70	64 (43%)	
≥ 70	29 (20%)	
Smoking status		
Non smoker	15 (10%)	
Former smoker	64 (43%)	
Current smoker	69 (47%)	
Histology		
Adenocarcinoma	109 (74%)	
Non-adenocarcinoma	39 (26%)	
Stage		
IIIb	17 (11%)	
IV	131 (89%)	
Number of metastasis sites		
≤ 2	111 (75%)	
> 2	37 (25%)	

567 **Table S2.** Univariate analysis of overall survival (OS)

	<i>Median [95% CI] (months)</i>	<i>Log-rank P-value</i>	<i>HR [95% CI]</i>	<i>P-value</i>
Monocytosis (N=128)				
< 0.8 x 10 ⁹ /L	16.9 [14.4 ; 23.5]	0.010	1 (ref)	0.011
≥ 0.8	11.6 [7.0 ; 14.3]		1.77 [1.14 ; 2.73]	
Lymphopenia (N=128)				
< 1 x 10 ⁹ /L	12.5 [6.0 ; 16.9]	0.325	1 (ref)	0.327
≥ 1	15.2 [11.0 ; 19.4]		0.78 [0.48 ; 1.28]	
Neutrophilia (N=128)				
< 7.5 x 10 ⁹ /L	19.6 [15.2 ; 25.9]	<0.001	1 (ref)	<0.001
≥ 7.5	10.8 [6.9 ; 13.2]		2.36 [1.51 ; 3.69]	
Leukocytosis (N=128)				
< 10 x 10 ⁹ /L	19.8 [15.4 ; 27.3]	<0.001	1 (ref)	<0.001
≥ 10	11.0 [6.9 ; 13.1]		2.41 [1.52 ; 3.80]	
LDH (N=108)				
< 248 U/L (ULN)	15.4 [13.1 ; 20.6]	0.006	1 (ref)	0.008
≥ 248 U/L	10.8 [6.0 ; 14.4]		2.25 [1.24 ; 4.08]	
dNLR* (N=128)				
≤ 3	15.4 [12.4 ; 20.6]	0.033	1 (ref)	0.035
> 3	12.5 [7.6 ; 15.5]		1.59 [1.03 ; 2.45]	

568 *dNLR = [Neutrophils/(Leukocytes-Neutrophils)]

569

570 **Table S3.** Univariate analysis of progression-free survival (PFS)

	<i>Median [95% CI] (months)</i>	<i>Log-rank P-value</i>	<i>HR [95% CI]</i>	<i>P-value</i>
Monocytosis (N=128)				
< 0.8 x 10 ⁹ /L	6.4 [5.1 ; 8.1]	0.091	1 (ref)	0.095
≥ 0.8	5.6 [3.7 ; 6.7]		1.38 [0.95 ; 2.01]	
Lymphopenia (N=128)				
< 1 x 10 ⁹ /L	5.6 [3.1 ; 8.5]	0.475	1 (ref)	0.479
≥ 1	6.0 [5.0 ; 6.9]		0.85 [0.54 ; 1.34]	
Neutrophilia (N=128)				
< 7.5 x 10 ⁹ /L	6.7 [5.0 ; 8.5]	0.020	1 (ref)	0.022
≥ 7.5	5.6 [4.3 ; 6.7]		1.56 [1.07 ; 2.28]	
Leukocytosis (N=128)				
< 10 x 10 ⁹ /L	6.7 [4.3 ; 8.9]	0.072	1 (ref)	0.075
≥ 10	5.6 [4.4 ; 6.7]		1.42 [0.97 ; 2.09]	
LDH (N=108)				
< 248 U/L (ULN)	5.9 [5.0 ; 6.9]	0.578	1 (ref)	0.582
≥ 248 U/L	6.8 [3.4 ; 7.8]		1.16 [0.69 ; 1.95]	
dNLR (N=128)				
≤ 3	5.9 [4.3 ; 7.1]	0.805	1 (ref)	0.807
> 3	6.6 [4.8 ; 7.0]		1.05 [0.72 ; 1.53]	

571 *dNLR = [Neutrophils/(Leukocytes-Neutrophils)]

572

573 **Table S4:** Clinical characteristics according to NKp30 status (N=148).

	<i>NKp30_ter: 1</i> <i>N=49</i>	<i>NKp30_ter: 2</i> <i>N=49</i>	<i>NKp30_ter: 3</i> <i>N=50</i>	<i>Total</i> <i>N=148</i> (%)	<i>P-value</i> <i>Chi² test</i>
Sex					
Male	32 (65%)	29 (59%)	37 (74%)	98 (66%)	0.293
Female	17 (35%)	20 (41%)	13 (26%)	50 (34%)	
Age at inclusion in MSN-Lung study (years)					
<i>N</i>	49	49	50	148	
Median	65	60	60	62	
Range	30 ; 83	34 ; 80	37 ; 81	30 ; 83	
Age at inclusion					
< 60 years	12 (24%)	21 (43%)	22 (44%)	55 (37%)	0.047
60-69 year	21 (43%)	22 (45%)	21 (42%)	64 (43%)	
≥ 70 year	16 (33%)	6 (12%)	7 (14%)	29 (20%)	
Smoking status					
Non smoker	5 (10%)	5 (10%)	5 (10%)	15 (10%)	0.488
Ex smoker	20 (41%)	26 (53%)	18 (36%)	64 (43%)	
Current smoker	24 (49%)	18 (37%)	27 (54%)	69 (47%)	
Histology					
Adenocarcinoma	39 (80%)	35 (71%)	35 (70%)	109 (74%)	0.507
Non-adeno	10 (20%)	14 (29%)	15 (30%)	39 (26%)	
Stage					
Stage IIIb	7 (14%)	4 (8%)	6 (12%)	17 (11%)	0.630
Stage IV	42 (86%)	45 (92%)	44 (88%)	131 (89%)	
Number of metastasic sites					
< 3	30 (61%)	39 (80%)	42 (84%)	111 (75%)	0.022
≥ 3	19 (39%)	10 (20%)	8 (16%)	37 (25%)	

575 **Table S5:** Blood parameters according to NKp30 status (N=148).

	<i>NKp30_ter:1</i> N=49	<i>NKp30_ter:2</i> N=49	<i>NKp30_ter:3</i> N=50	<i>Total</i> N=148 (%)	<i>P-value</i> [§] <i>Chi² test</i>
Monocytosis					
< 0.8 x 10 ⁹ /L	13 (33%)	30 (67%)	25 (58%)	68 (53%)	0.005
≥ 0.8	27 (68%)	15 (33%)	18 (42%)	60 (47%)	
Missing	9	4	7	20	
Lymphopenia					
< 1 x 10 ⁹ /L	8 (20%)	9 (20%)	9 (21%)	26 (20%)	0.992
≥ 1	32 (80%)	36 (80%)	34 (79%)	102 (80%)	
Missing	9	4	7	20	
Neutrophilia					
< 7.5 x 10 ⁹ /L	15 (38%)	22 (49%)	22 (51%)	59 (46%)	0.412
≥ 7.5	25 (63%)	23 (51%)	21 (49%)	69 (54%)	
Missing	9	4	7	20	
Leukocytosis					
< 10 x 10 ⁹ /L	15 (38%)	19 (42%)	18 (42%)	52 (41%)	0.888
≥ 10	25 (63%)	26 (58%)	25 (58%)	76 (59%)	
Missing	9	4	7	20	
LDH (U/L)					
< 248 (ULN)	19 (63%)	35 (85%)	32 (86%)	86 (80%)	0.033
≥ 248	11 (37%)	6 (15%)	5 (14%)	22 (20%)	
Missing	19	8	13	40	

576 [§] Chi² test *P-value* does not take into account missing values

577

578 **Table S6:** Blood parameters according to sBAG6 status (*N*=65).

	<i>sBAG6ter: 1</i> <i>N=21</i>	<i>sBAG6_ter: 2</i> <i>N=22</i>	<i>sBAG6_ter: 3</i> <i>N=22</i>	<i>Total</i> <i>N=65</i> (%)	<i>P-value</i> <i>Chi² test</i>
Monocytosis					
< 0.8 x 10 ⁹ /L	10 (53%)	5 (33%)	9 (45%)	24 (44%)	0.530
≥ 0.8	9 (47%)	10 (67%)	11 (55%)	30 (56%)	
Missing	2	7	2	11	
Lymphopenia					
< 1 x 10 ⁹ /L	1 (5%)	3 (20%)	7 (35%)	11 (20%)	0.070
≥ 1	18 (95%)	12 (80%)	13 (65%)	43 (80%)	
Missing	2	7	2	11	
Neutrophilia					
< 7.5 x 10 ⁹ /L	13 (68%)	5 (33%)	5 (25%)	23 (43%)	0.016
≥ 7.5	6 (32%)	10 (67%)	15 (75%)	31 (57%)	
Missing	2	7	2	11	
Leukocytosis					
< 10 x 10 ⁹ /L	9 (47%)	3 (20%)	7 (35%)	19 (35%)	0.252
≥ 10	10 (53%)	12 (80%)	13 (65%)	35 (65%)	
Missing	2	7	2	11	
LDH (U/L)					
< 248 (ULN)	10 (71%)	8 (67%)	15 (88%)	33 (77%)	0.339
≥ 248	4 (29%)	4 (33%)	2 (12%)	10 (23%)	
Missing	7	10	5	22	

579

580

581 **Table S7:** Flow cytometry Antibody.

<i>Antibodies</i>	<i>Supplier</i>	<i>Clone</i>
CD16-FITC	Beckman	3G8
	Coulter	
NKp30-PE	Beckman	Z25
	Coulter	
CD56-PC7	Beckman	N901(HLDA
	Coulter	6)
NKG2D-APC	Beckman	ON72
	Coulter	
CD45-AF750	Beckman	J33
	Coulter	
CD3-VB	Miltenyi	BW264/56
TCR pan g/d- FITC	Beckman	IMMU510
	Coulter	
NKp44-PE	Beckman	Z231
	Coulter	
HLA-DR-ECD	Beckman	Immu-357
	Coulter	
NKp30-PC5.5	Beckman	Z25
	Coulter	

NKp46-PC7	Beckman	BAB281
	Coulter	
CD3-AA700	Beckman	UCHT1
	Coulter	
CD16 AA750	Beckman	3G8
	Coulter	
CD56 BV421	Sony	HCD56
CD8 KrOr	Beckman	B9.11
	Coulter	
CD69 ECD	Beckman	TPL55.3
	Coulter	

582

583

584 **Table S8:** qRT-PCR primers and TaqMan probes sequences.

<i>Name</i>	<i>Sequence</i>
NKp30-EC	5'-TTTCCTCCATGACCACCAGG-3'
(Forward):	
NKp30-EX4I	5'-TTCCCATGTGACAGTGGCATT-3'
(Reverse):	
NKp30-EX4II	5'-CGGAGAGAGTAGATTTGGCATATT-3'
(Reverse)	
NKp30-EX4III	5'-GGACCTTTCCAGGTCAGACATT-3'
(Reverse)	
NKp30-Probe	5'- (6FAM)TGGTGGAGAAAGAACATCCTCAGCTAGGG(TAM)- 3'
β2M-F (Forward)	5'-GATGAGTATGCCTGCCGTGT-3'
β2M-R (Reverse)	5'-AATTCATCCAATCCAAATGCG-3'
β2M-Probe	5'-(6FAM)AACCATGTGACTTTGTCACAGCCCAA(TAM)-3'
NCR3	Hs00394809_m1 (Applied biosystem, Life Technologies)
NCR1	Hs00183118_m1 (Applied biosystem, Life Technologies)
NCR2	Hs00183113_m1 (Applied biosystem, Life Technologies)

585

586

587