

Correlation Between Natural Killer Cell Activity and Treatment Effect in Patients with Disseminated Cancer¹



Torben Frøstrup Hansen^{*,†,‡}, Line Nederby[§],
Ahmed H. Zedan^{*,†}, Inge Mejlholm^{*},
Jon R. Henriksen^{*,†}, Karina D. Steffensen^{*,†,‡},
Caroline B. Thomsen^{*,†,‡}, Louise Raunkilde^{*},
Lars Henrik Jensen^{*,†,‡} and Anders Jakobsen^{*,†,‡}

^{*}Department of Oncology, Vejle Hospital, Vejle, Denmark; [†]Institute of Regional Health Research, University of Southern Denmark, Denmark; [‡]Danish Colorectal Cancer Center South, Vejle Hospital, Vejle, Denmark; [§]Department of Clinical Immunology and Biochemistry, Vejle Hospital, Vejle, Denmark

Abstract

INTRODUCTION: The aim of the present study was to analyze the possible correlation between Natural Killer (NK) cell activity as measured by the NK Vue assay and treatment efficacy in patients with disseminated cancer. **MATERIALS AND METHODS:** The study included four trials encompassing palliative treatment, i.e. one trial on prostate- and ovarian cancer, respectively, and two trials on colorectal cancer. The current results are based on 93 patients with mature data on treatment effect. Blood samples were collected at baseline and prior to each treatment cycle into NK Vue. Following 24 hours of stimulation the level of interferon-gamma (IFN γ) in the plasma was measured as a surrogate for NK cell activity. **RESULTS:** The relationship between NK cell activity and treatment response was similar across tumor types and treatment. The IFN γ either remained at or dropped to an abnormal level (<200 pg/mL) during treatment in group 1 (n = 35). In group 2 (n = 30) the level remained within a normal range (>200 pg/mL), while in group 3 (n = 28) it increased from an abnormal to a normal level. The response rate was 14%, 47%, and 82%, respectively, $P < .001$. The median progression free survival was 2.6 months (95% confidence interval (CI) 2.1–3.9), 10.0 months (95% CI 6.5–11.1), and 8.3 months (95% CI 6.5–8.7), respectively, $P < .001$ (log-rank). **CONCLUSION:** Patients lacking the ability to mount an immune response during the first 2 months of treatment have a poor prognosis, and their clinical benefit of the treatment is questionable.

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Introduction

Natural killer (NK) cells are part of the innate immune system and play an important role in the initial response to infected as well as malignant cells [1]. They are capable of killing target cells without prior antigen presentation and take part in coordinating the adaptive immune response through the release of inflammatory cytokines and chemokines, of which interferon- γ (IFN γ) is of great importance [1,2].

Several studies have demonstrated debilitated functions of NK cells in patients with cancers as compared to healthy controls [3], including colorectal cancer [4–6], ovarian cancer [7], and prostate cancer [8–10]. It has also been postulated that the activity level of NK cells may provide an estimate of disease activity and relate to the escape mechanisms from immune surveillance [11]. Together, these findings designate the activity level of NK cells as a potential

biomarker with implications as a tool for monitoring the immune system during treatment. More importantly, the activity level may provide clinically relevant information as to the effectiveness of a

Address all correspondence to: Torben Frøstrup Hansen, Department of Oncology, Vejle Hospital, Beriderbakken 4, 7100, Vejle, Denmark. E-mail: torben.hansen@rsyd.dk

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given treatment at a given time in a patient with cancer. The current standard methodologies such as ^{51}Cr -release assays and in vitro cytokine stimulations are effective but cumbersome, which ultimately has limited the use of NK cell activity as a biomarker in the clinical setting. With the recent introduction of a simple assay for measuring NK cell activity this parameter may be clinically applicable since reliable biomarkers for treatment efficacy has been called for through decades.

The aim of the present study was to analyze the possible correlation between NK cell activity as measured by the NK Vue assay and treatment efficacy in patients with metastatic cancer.

Material and Methods

This study is reported in accordance with REMARK [12].

Patient Populations and Treatment

This translational study included patients from four ongoing biomarker studies encompassing palliative treatment in patients with metastatic disease (castration resistant prostate cancer, ovarian cancer, and colorectal cancer). The current results are based on 93 patients with mature data on treatment effect. Patients were enrolled between April 2016 and September 2017.

In brief, all patients had disseminated disease and were treated with palliative oncologic treatment according to national guidelines and protocol specifications, i.e., chemotherapy regimens in the ovarian- and colorectal cancer studies and enzalutamide in the prostate cancer study. Treatment continued until disease progression or unacceptable toxicity, but treatment-free intervals were accepted if requested by the patient. Response evaluation was based on clinical examination (including CA-125 in ovarian cancer) and CT scans of the chest and abdomen (plus bone scan in prostate cancer) every 8–12 weeks (depending on regimen) and assessed according to the RECIST 1.1 criteria [13]. Responding patients were classified as having complete response (CR) or partial response (PR), while non-responding patients were classified with stable disease (SD) or progressive disease (PD).

The main selection criteria included; histologically verified diagnosis, evaluable disease by imaging, age ≥ 18 years, performance status (PS) ≤ 2 , life expectancy ≥ 3 months, adequate bone marrow and organ function, at least two treatment cycles received, and a minimum of two evaluations (CT scans) during treatment unless progressive disease (PD) recorded at first evaluation. The exclusion criteria were; severe medical co-morbidity, other malignant disease within 5 years prior to study enrollment, except basocellular or squamous skin cancer and carcinoma in situ cervicis uteri.

All data were recorded according to good clinical practice. The studies, and the present translational research, were approved by the Regional Committee on Health Research Ethics for Southern Denmark (S-20150185, S-20150103, S-20160029, and S-20160049) and the Danish Data Protection Agency. Written informed consent was obtained from all patients enrolled in the study.

Sampling and NK Cell Activity Analyses

Peripheral blood was sampled before initiation of treatment (baseline) and prior to each treatment cycle until disease progression. The basic principle behind the NK Vue test is the sampling of peripheral blood into a patented test tube, incubation, and estimation based on the ELISA. An illustration can be assessed at <http://nkvue.com/en/sub/product/introduce.php>. In specific, we did the following: One milliliter of venous blood (whole blood) was drawn from the

antecubital area into NK Vue tubes (ATGen, Seongnam-si, South Korea) and placed in an incubator at 37 °C within 15 minutes of sampling. Following 24 hours of stimulation the level of IFN γ in the plasma was measured by the NK Vue ELISA (ATGen ELISA) as a surrogate marker of NK cell activity. The linear dynamic range of the ELISA was 65–2000 pg/mL, the limit of quantification was 65 pg/mL, and the limit of detection was 15 pg/mL. Samples with test results above the upper limit were diluted 1:10 and reanalyzed. Samples with activity levels below 15 pg/mL were recorded as 15 pg/mL. The in-house analytical coefficients of variation (CV) of the ELISA were $<10\%$ for Intra-Assay and $<12\%$ for Inter-Assay. A cut-off of 200 pg/mL differentiates between abnormal (<200 pg/mL) and normal (≥ 200 pg/mL) values (ATGen recommendation). Sampling and analyses were performed by staff unaware of patient outcome.

Prior to the study we performed duplicate analyses on 125 healthy individuals (supplementary Figure S1) indicating a valid test assay especially for test values in the range of 0–2,500 pg/mL corresponding to the majority of patients with cancer. We also tested whether the IFN γ test result corresponded to the number of NK cells. This was not the case for any of the three cancer types (supplementary Figure S2).

Statistics

We report median values with a 95% confidence interval (CI). The Wilcoxon rank sum test was used for comparison of median values. Differences between proportions were evaluated using Chi-Square Statistics. Progression free survival (PFS) was defined as the time from the first day of the first treatment cycle to the first documented tumor progression or death of any cause. The PFS data were censored in five cases due to liver surgery (patients from colorectal cancer trials only) with the date of the intervention as cut-off. Multiple comparisons were not adjusted for. All statistics were calculated using the NCSS statistical software (NCSS Statistical Software, Kaysville, UT 84037, USA, version 2007). P values $<.05$ were considered significant, and all tests were two-sided.

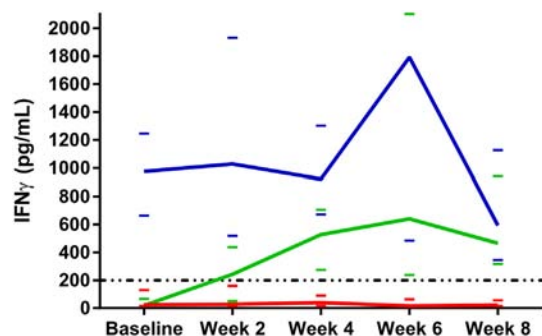


Figure 1. Illustration of median IFN γ values during the first 2 months of treatment. Group 1 is represented by the red curve, group 2 the blue, and group 3 the green curve. The dashed line marks the cut-off value of 200 pg/mL differentiating between abnormally low and normal test values. The small colored bars represent the 95% confidence intervals of the respective medians (for graphical reasons the upper limit of group 2 at week six is not depicted (7391 pg/mL)). There was no significant difference between groups 1 and 3 at baseline ($P = .364$), but at week 2 they were statistically different ($P = .019$). Group 2 differs significantly from both group 1 and 3 at baseline ($P < .001$ and $P < .001$) (Wilcoxon Rank-Sum test for difference in medians).

Results

Interpretation of the NK Cell Activity Test Results

The changes in NK cell activity during the first 2 months of treatment were similar across tumor types and treatments suggesting a classification into three groups. In group 1 IFN γ dropped to an abnormal level (<200 pg/mL) or remained at an abnormal level (n = 35). The test results in group 2 (n = 30) remained within a normal range (>200 pg/mL) while patients in group 3 (n = 28) were characterized by an increase of the NK cell activity from an abnormal level at baseline to normal values. These patterns are illustrated in Figure 1. The NK cell activity in group 2 differs significantly from group 1 at all sampling points ($P < .001$) whereas group 3 cannot be distinguished from group 1 at baseline but diverges after only two weeks of treatment ($P = .019$).

Patient Characteristics

The median follow-up (patients without progression) was 7.3 months and 54 patients had documented progression. Clinical and pathoanatomic characteristics are shown in Table 1. The baseline NK cell activity was significantly lower in the younger half of the patients, $P = .032$. Based on the fact that Group 1 and 3 cannot be distinguished from each other at baseline we compared the pathoanatomic characteristics with the division into the three groups. Table 2 shows that a Group 1 pattern was more common among the older half of the patients, prostate cancer, and patients with PS 1–2.

Treatment Efficacy

There was a significant relationship between response rate (RR) and the changes in NK cell activity during the first 2 months of

Table 1. Clinical and Pathoanatomic Characteristics, n = 93

Parameter	Combined Cohort	Baseline NK Cell Activity (pg/mL)	P
	N = 93 (%)	Median (95% Confidence Interval)	
Gender			
Male	50 (54)	141 (34–259)	.820
Female	43 (46)	107 (15–601)	
Age, median 68			
> 68	43 (46)	332 (48–721)	.032
≤ 68	50 (54)	53 (15–175)	
Line of treatment			
1	54 (58)	144 (34–259)	.943
2 or more	39 (42)	71 (17–663)	
Baseline LDH, median 214 U/L^a			
> 214	42 (49)	29 (15–175)	.054
≤ 214	43 (51)	199 (54–601)	
Disease			
Colorectal cancer	51 (55)	126 (17–259)	.669 ^b
Ovarian cancer	23 (25)	107 (15–663)	.335 ^c
Prostate cancer	19 (20)	212 (20–1524)	.126 ^d
Ethnicity			
Caucasian	93 (100)	139 (31–259)	
Performance status			
0	51 (55)	144 (39–547)	.284
1–2	42 (45)	48 (15–300)	
Smoking status^c			
Never or former smoker	75 (86)	142 (31–291)	.829
Active smoker	12 (14)	47 (15–740)	

NK, Natural killer; LDH, lactate dehydrogenase.

All P values refer to the Wilcoxon Rank-Sum test for difference in medians.

^a Eight patients missed values for comparisons.

^b Comparison between colorectal and ovarian cancer, ^c comparison between ovarian and prostate cancer, ^d comparison between colorectal and prostate cancer.

^e Data on smoking status was not available for six patients.

Bold P value highlights statistical significance.

Table 2. Clinical and Pathoanatomic Characteristics According to the Grouping, n = 93

Parameter	Grouping According to NK Cell Activity			P
	Group 1	Group 2	Group 3	
Gender				
Male	22	12	16	.167
Female	13	18	12	
Age, median 68				
> 68	20	16	7	.025
≤ 68	15	14	21	
Line of treatment				
1	21	14	19	.252
2 or more	14	16	9	
Baseline LDH, median 214 U/L^a				
> 214	20	10	16	.084
≤ 214	14	20	12	
Disease				
Colorectal cancer	15	15	21	.033
Ovarian cancer	8	10	5	
Prostate cancer	12	5	2	
Performance status				
0	8	21	22	<.001
1–2	27	9	6	
Ethnicity				
Caucasian	35	30	28	-
Smoking status				
Never or former smoker	29	25	21	.914
Active smoker	4	4	4	

NK, natural killer; LDH, lactate dehydrogenase.

P values refer to chi-square statistics.

^a One patient missed values for comparisons.

treatment. The RR in group 1, 2, and 3 was 14% (5/35), 47% (14/30), and 82% (23/28), respectively, $P < .001$.

The association with PFS was also significant in the three groups, $P < .001$ (Figure 2). The median PFS was 2.6 months (95% confidence interval (CI) 2.1–3.9), 10.0 months (95% CI 6.5–11.1), and 8.3 months (95% CI 6.5–8.7), in Group 1, 2, and 3, respectively. Table 3 shows the results of the Cox regression simple and multiple analyses. Variables with $P < .15$ in the simple analysis were included in the multiple analysis. An independent impact on PFS was shown for the following variables; line of treatment, PS, and the NK cell activity groups. The latter demonstrated a HR of 4.368 (95% CI, 1.942–9.824), $P < .001$, in patients with a group 1 pattern.

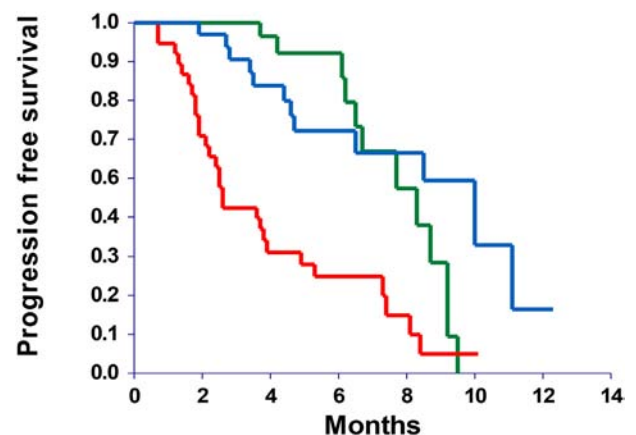


Figure 2. Kaplan–Meier progression free survival curves. Group 1 (N = 35) is represented by the red curve, group 2 (N = 30) the blue, and group 3 (N = 28) the green curve. The difference between the curves is significant, $P < .001$ (log-rank).

Table 3. Cox Regression Analysis of Progression Free Survival, $n = 92$ in the Multiple Analysis

Parameter	Simple Analysis		Multiple Analysis	
	HR (95% CI)	<i>P</i>	HR (95% CI)	<i>P</i>
Gender				
Female	1		1	.237
Male	0.651 (0.378–1.120)	0.121	0.634 (0.298–1.349)	
Age, median 68				
≤ 68	1			
> 68	0.913 (0.528–1.576)	0.742		
Line of treatment				
1	1		1	
2 or more	1.786 (1.038–3.075)	0.036	2.450 (1.174–5.110)	.017
Baseline LDH, median 214 U/L*				
≤ 214	1		1	
> 214	1.554 (0.884–2.734)	0.126	0.943 (0.491–1.812)	.860
Disease				
Colorectal cancer	1			
Prostate cancer	0.546 (0.237–1.258)	0.155		
Ovarian cancer	1.190 (0.648–2.184)	0.575		
Smoking status				
Never or former smoker	1			
Active smoker	0.690 (0.309–1.543)	0.366		
Performance status				
0	1		1	
1–2	4.340 (2.308–8.162)	<0.001	2.358 (1.175–4.731)	.016
NK cell activity Group				
Group 3	1		1	
Group 2	0.770 (0.339–1.745)	0.531	0.680 (0.292–1.582)	.370
Group 1	3.614 (1.823–7.165)	<0.001	4.368 (1.942–9.824)	<.001

HR, Hazard ratio; LDH, lactate dehydrogenase; NK, natural killer.

Discussion

We analyzed the possible correlation between NK cell activity measured in the blood, by the NK Vue assay and treatment effect in patients with metastatic cancers. Changes in NK cell activity during the first 2 months of treatment were similar across tumor types and treatment and a highly significant relationship with treatment effect was demonstrated.

We have recently conducted a technical study of the NK Vue assay concluding that NK cells were the predominant source of IFN γ in the assay but T cells and natural killer T cells also contributed [14]. Having confirmed the ability of the assay to estimate NK cell activity we applied it in the clinical setting. Our present results shows that the changes in NK cell activity during the first 2 months of treatment seem to divide patients into three distinct groups regardless of tumor type and treatment. Patients with a group 1 or 3 pattern were characterized by an abnormally low NK cell activity at baseline. During the course of treatment patients in group 3 were able to mount an immune response upon stimulation while the NK cell activity in group 1 remained suppressed throughout the course of treatment. On the other hand, the immune response of patients in group 2 seemed unaffected by the disseminated cancer, thus displaying an NK cell activity within the normal range at all sampling points. The interpretation of this pattern (group 2) raises questions as to why the disease in one third of the patients appears to be unaffected by a seemingly well-functioning immune response.

The baseline NK cell activity was significantly lower in the younger patients and to some extent also low in patients with the highest baseline LDH levels, though non-significant. This observation seems plausible and may reflect 1) the often more aggressive phenotype of cancer in young patients and 2) the often greater tumor burden associated with a high level of LDH [15]. Caution should, however, be paid to the age distribution in this mixed cohort. Since the patients with prostate cancer were significantly older than the patients in the

remaining three study cohorts, a disease specific impact on this comparison cannot be ruled out.

A highly significant relationship was demonstrated between the NK cell activity patterns and treatment efficacy assessed by RR and PFS. Patients in group 1 had a significantly poorer prognosis than those in the other two groups with more than half the patients progressed at first evaluation. The unfavorable outcome in these patients was further highlighted with an HR of 4.4 in the multiple Cox analysis. The treatment efficacy did not differ between groups 2 and 3.

Cancer cells may develop a variety of immune evasive strategies to escape IFN γ -dependent immune responses by downregulation of IFN γ signaling pathways, induction of signaling pathways promoting tumor growth, and production of anti-inflammatory molecules [16]. The resistance to IFN γ may be more pronounced in group 2, which could in fact explain the high test values, the modest RR, and a PFS comparable to patients in group 3.

This study is so far the first to evaluate the relationship between NK cell activity estimated by the NK Vue assay and treatment effect in patients with disseminated cancer. Consequently, a direct comparison with existing literature may be biased due to methodological differences. Similarities, however, can be drawn to a number of studies. In 2009, Ménard et al. published data on maturing dendritic cell-dependent IFN γ production by NK cells in 56 patients with gastrointestinal stromal tumors (GIST) treated with imatinib [17]. Similar to our study they assessed IFN γ levels at baseline and after 2 months of treatment. Immunological responders, defined by an increase in IFN γ above baseline values after 2 months, demonstrated a significantly better PFS compared to the non-immunological responders, i.e. decreased IFN γ values, which clearly compares to our results on group 3 and group 1 patients. Likewise, our data paralleled findings by Pasero and colleagues who demonstrated that effective NK cells in patients with newly diagnosed metastatic prostate cancer were associated with a longer time to castration resistance and overall survival [18]. Also, there were

obvious similarities between our results and previous work by Garzetti et al. who followed 17 patients with ovarian cancer stage III-IV by analyzing NK cell activity at baseline (before surgery), during the course of cisplatin-containing chemotherapy, and during follow-up [19]. They demonstrated a significantly lower baseline NK cell activity in patients with subsequent disease recurrence compared to those who remained recurrence free, thus suggesting a prognostic relationship.

The modest sample size and the mixture of cancers and treatments in the current study may have influenced some of our conclusions on the relationship between NK cell activity and clinicopathological characteristics. However, the compelling relationship between test results and treatment effect substantiates the potential of this assay in different settings although the exact interpretation awaits final results from the individual tumor types.

Conclusion

The results suggest a correlation between NK cell activity and treatment effect across different solid tumor types and treatments. Patients lacking the ability to mount an immune response during the first 2 months of treatment have a poor prognosis, and the clinical benefit of the anti-cancer treatment is questionable. Assessment of NK cell activity may represent an additional way of monitoring treatment effect in oncology practice.

Supplementary data to this article can be found online at <https://doi.org/10.1016/j.tranon.2019.04.002>.

Disclosure Statement

AJ has received research funding from ATGen Co. Ltd. The company had no influence on the study design, analyses, data interpretation, manuscript preparation, or submission process.

Author Contribution

All authors participated in conceiving and designing the experiments. TFH performed statistical analyses of the data and drafted the manuscript. All authors were responsible for collection and assembly of data. LN supervised the NK cell activity assessment. All authors discussed the results, commented, and approved the final version of the manuscript.

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