Introduction: Immune responses within the tumor depend on the ability of leukocytes to migrate from peripheral circulation into the local microenvironment. This process is controlled by mechanisms that guide leukocytes to the side of inflammation, allowing them to cross vascular endothelial barrier. Monocytes/macrophages are the predominant population of leukocyte infiltrate of many tumors, including gastric cancer. However, to date mechanisms that control monocyte trafficking to the side of tumor growth are not fully elucidated.

Aim of the study: In this study we aimed to evaluate transmigratory potential of peripheral blood monocytes from gastric cancer patients.

Material and methods: By using multicolor flow cytometry we assessed expression of \( \beta_1 \)- and \( \beta_2 \)-integrins on peripheral blood monocytes from gastric cancer patients.

Results: We found increased frequencies of VLA-4 and VLA-6 expressing monocytes and increased expression of analyzed \( \beta_2 \)-integrins in gastric cancer patients when compared to age matched controls.

Conclusions: In summary, this study revealed that gastric cancer increases transmigratory potential of peripheral blood monocytes.

Key words: gastric cancer, monocytes, \( \beta_2 \)-integrins, VLA-4, VLA6.
rial Centre of Oncology in Bydgoszcz (Poland), were enrolled to the study (Table 1). None of the patients received chemotherapy and radiotherapy before or was subjected to surgery or blood transfusion for at least six month before blood acquisition. Furthermore, none of the patients showed any clinical or cellular sings on ongoing infection. Peripheral blood was collected upon the approval of the Bioethical Committee of the Collegium Medicum in Bydgoszcz. Each participant was familiarized with the objectives of the study and expressed written consent.

Flow cytometry
100 μl of fresh heparin-anticoagulated blood was stained with panel of mouse anti-human monoclonal antibodies (Table 2). Stain-then-lyse protocol was used as previously described [12]. Appropriate fluorescence-minus-one (FMO) and isotype controls were used for every staining for setting compensation and to assure correct gating. Samples were analyzed by using FACScan flow cytometer (Becton Dickinson) and at least 40 000 events were collected. Next, collected data were analyzed by using FlowJo version 7.6.1. (TreeStar). Used gating strategy is presented on Fig. 1.

Statistics
Statistical analysis was carried out using GraphPad Prism 6 software (GraphPad Software). U Mann-Whitney test was used. The differences were considered statistically significant at \( p < 0.05 \). The results are presented as median (interquartile range).

Results
Due to the constitutive expression of analyzed \( \beta_2 \)-integrins on the surface of monocytes we analyzed their expression levels. First we found significant increase of CD11a and CD11b expression in gastric cancer patients when compared to normal donors. Additionally, we found no differences in CD11c and CD18 expression level. Next, we found that gastric cancer increase frequencies of CD49d (\( \alpha_4 \) subunit of VLA-4 integrin) and CD49f (\( \alpha_6 \) subunit of VLA-6 integrin) expressing monocytes. Interestingly, we did not observed any differences in expression level of above mentioned molecules.

Discussion
Integrins are membrane glycoproteins controlling numerous physiological processes, including cell adhesion, chemotaxis, and phagocytosis [13]. \( \beta_2 \)-integrins are heterodimeric receptor proteins consisting of \( \alpha \) and \( \beta \) subunits linked by sulphide bridges. All receptors shear common \( \beta_2 \)-chain (CD18) and differ in \( \alpha \) chain variants, namely \( \alpha L – CD11a; \alpha M – CD11b; \alpha X – CD11c \). They are involved in direct adhesion of leukocytes to endothelial cells [14, 15]. Similarly, \( \beta_1 \)-integrins shear common \( \beta_1 \) chain (CD29) and differ in \( \alpha \) subunits. To date, at least 10 different \( \alpha \) chains were discovered including CD49d and CD49f expressed on monocytes (Fig 2). In contrast to \( \beta_2 \)-integrins, the latter represent a group of protein receptor responsible for cell interactions with extracellular matrix [13]. Here, we found that gastric cancer increase frequencies of both VLA-4 and VLA-6 expressing monocytes (Fig 3). Interestingly, Jin et al. showed that VLA-4 is playing leading role in monocyte transmigration to tumor microenvironment [16]. However, in some contrast to previous studies by Zhang et al., they found that this process occurred in the \( \alpha M \beta 2 \) (CD11b/CD18) integrin independent manner [16, 17]. It seems, that \( \beta_2 \)-integrins may play supportive role in monocyte transmigration process and observed upregulation of CD11a and CD11b expression is a consequence of monocyte activation and their inflammatory phenotype [18]. Notably, in our previous report we found that gastric cancer patients showed increased frequencies of inflammatory (activated) monocytes, namely intermediate (CD14++CD16+) and non-classical (CD14+CD16++) cells [12]. Interestingly, VLA-4 support not only transmigration

### Table 1. Clinical characteristics of study population

| Parameter | Gastric cancer | Normal |
|-----------|----------------|--------|
| n         | 40             | 17     |
| Mean age (range) | 62.79 (30-86) | 58.35 (49-68) |
| Gender (female/male) | 13/27 | 8/9 |
| Stage according to AJCC (frequencies of all) | | |
| Stage I   | 12 (30%)       |        |
| Stage II  | 2 (5%)         |        |
| Stage III | 14 (35%)       |        |
| Stage IV  | 12 (30%)       |        |

AJCC – American Joint Committee on Cancer

### Table 2. Characteristics of monoclonal antibodies used in the study

| Name | Clone | Isotype | Format | Manufacturer |
|------|-------|---------|--------|--------------|
| CD14 | MoP9  | IgG2b   | PerCP  | Becton Dickinson |
| CD49d (VLA-4 \( \alpha \) subunit) | 9F10 | IgG1 | FITC | Becton Dickinson |
| CD49f (VLA-6 \( \alpha \) subunit) | 1.BB.460 | IgG2b | FITC | Becton Dickinson |
| CD11a | G43-25B | IgG2a | FITC | Becton Dickinson |
| CD11b | 1.BB.189 | IgG1 | FITC | Santa Cruz Biotechnology |
| CD11c | B-Ly6 | IgG1 | PE | Becton Dickinson |
| CD18 | 6.7 | IgG1 | PE | Becton Dickinson |

PerCP – peridinin chlorophyll protein complex; FITC – fluorescein isothiocyanate; PE – phycoerythrin
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of inflammatory monocytes but also the transition of macrophages from classically activated (M1) to pro-tumoral (M2) cells, by Rac2 activation [19]. Therefore, increased frequencies of VLA-4 expressing monocytes may be a consequence of their non-classical activation. Furthermore, VLA-6 was shown to support tumor growth and angiogenesis by promoting tumor infiltration of Tie-2 expressing monocytes and macrophages (TEMs) [20]. In summary, we showed here that peripheral blood monocytes from gastric cancer patients possess high transmigratory potential and are sensitive for non-classical polarization.

This study was partially supported by the grant for young scientists (CM UMK no. 7/WF/2013) from the Nicolaus Copernicus University; AE, MM are supported by funds from Leading National Scientific Center in Białystok of Medical University of Białystok.

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Fig. 1. Gating strategy for monocytes. Representative FACS plots demonstrating gating strategy to evaluate CD49d (VLA-4 α subunit), CD49f, CD11a, CD11b, CD11c and CD18 expressing monocytes and fluorescence intensity of analyzed receptors.
Fig. 2. β2-integrin expression in peripheral blood monocytes. Summary of analyses of (A) CD11a, (B) CD11b, (C) CD11c and (D) CD18 expression on CD14+ cells. Data are presented as median and interquartile range.

Fig. 3. VLA-4 and VLA-6 expression in peripheral blood monocytes. Summary of analyses of (A) VLA-4 and (B) VLA-6 expressing monocytes and (C) VLA-4 and (D) VLA-6 expression on CD14+ cells. Data are presented as median and interquartile range.
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