Dendritic cells transfected with a polyepitope DNA construct stimulate an antitumor cytotoxic response in various tumors

VASILY KURILIN[1], EKATERINA KULIKOVA[1], JULIA SHEVCHENKO[1], JULIA LOPATNIKOVA[1], IRINA OBLEUKHOVA[1], JULIA KHANTAKOVA[1], AMIR MAKSYUTOV[1,2], MARIA KUZNETSOVA[1], ALEXANDER KHRISTIN[3], NATALYA KIRYSHINA[4], VADIM KOZLOV[4], SERGEY SIDOROV[3], ANDREY SOKOLOV[3], ALEXANDER VITSIN[3], HIROSHI SHIKU[1,5] and SERGEY SENNIKOV[1]

[1]Federal State Budgetary Scientific Institution 'Research Institute of Fundamental and Clinical Immunology', Novosibirsk 630099; [2]State Research Center of Virology and Biotechnology 'Vector', Koltsovo 630559; [3]State Clinical Hospital No. 1, Novosibirsk, Novosibirsk 630075; [4]Novosibirsk Regional Clinical Oncological Center, Novosibirsk, Novosibirsk 630108, Russian Federation; [5]Mie University Graduate School of Medicine, Tsu, Mie 514-8507, Japan

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Abstract. Dendritic cells (DCs) loaded with tumor-associated antigens (TAAs) are known to be crucial for the antitumor response and are still included in various treatment regimens in cancer immunotherapy research. In the present study, a cell-based protocol was evaluated, involving the use of original DNA constructs encoding the wide range of TAA epitopes expressed on different epithelial cancers. The constructs were transfected into *in vitro*-generated DCs of patients with various types of cancer, including breast, colorectal and non-small cell lung cancer. The direct cytotoxicity assay of effector cells, activated with the transfected DCs, revealed a significant increase in cytotoxicity against autologous tumor cells. The use of DNA constructs encoding a large number of TAAs for insertion into DCs *in vitro*, aiming to activate a T-cell response may prove to be a reliable and unified approach for immunotherapy and for the prevention of relapse in patients with epithelial cancers.

Introduction

Novel technologies in cancer immunotherapy have made significant strides with the emergence of therapeutic approaches, including blocking checkpoint molecules, including cytotoxic T-lymphocyte (CTL)A-4, PD-1, TIM-3, LAG-3, TIGIT, BTLA (1-5) and genetically modifying effector cells, including CAR-T and TCR-T (6,7). Nevertheless, overcoming the limitations appearing and the cost reduction of such technologies is a subject that remains to be elucidated In addition, the study of all possible therapeutic approaches to the induction of an immune response is still highly relevant (8,9). To overcome the negative effects of the tumors on the formation of immune response, cytokines including IL-12, IL-18 and IL-2 (10,11), antibodies against immunosuppressive molecules (CTLA-4 and PD-1) expressed on the surface of tumor cells (12) and enzyme inhibitors (indoleamine 2,3-dioxygenase 1, prostaglandin-endoperoxide synthase 2) (13,14). The use of cellular immunotherapy is suggested at different stages of cancer development, in combination with the main complex antitumor therapy (15-18). The effectiveness of cellular immunotherapy is dependent on the stage of the disease, the type of tumor and the severity of systemic immunosuppression (19).

One of the most important aspects of efficient immune response formation is proper antigen presentation, in order to enable an endogenous antigen-specific cytotoxic T-cell response (20,21). Dendritic cells (DCs) play a crucial role in the activation of the antitumor immunity (22-24). The functional activity of DCs has been demonstrated to be significantly reduced in cancer patients (24-26). In cancer, the ability of DCs to capture tumor antigens and present them to the T-cells, as well as to mount an effective cellular response, is impaired (27,28). The main reason is considered to be the impairment of the DC maturation process (7), as well as the T-cell activation mechanisms (27,28).

The activation of T cell-related endogenous immune response *ex vivo*, outside the influence of the immunosuppressive tumor microenvironment, has been demonstrated to help succeed in obtaining efficient T-cells for tumor elimination when administered to patients as part of combination therapy (29,30). Several studies have confirmed the effectiveness of DC antigen loading using DNA constructs (31-33). In relation to this, the search for an effective combination of the

Correspondence to: Professor Sergey Sennikov, Federal State Budgetary Scientific Institution 'Research Institute of Fundamental and Clinical Immunology', Yadrintsevskaya 14, Novosibirsk 630099, Russian Federation
E-mail: sennikovsv@gmail.com

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aforementioned therapeutic approaches is of utmost scientific interest for further study and development of protocols for wide clinical use for immunotherapy in cancer patients.

Tumor-associated antigens (TAAs) are usually presented in a wide range of epithelial tumors, and each tumor can express a wide range of known antigens (34-37). Previously, the efficiency of *in vitro* generation antitumor immune response with the use of DCs transfected with DNA constructs encoding epitopes of particular tumor-associated antigen determinants was demonstrated by the authors (38,39). In the present study, this therapeutic approach was optimized and the hypothesis about whether the efficiency of using genetic constructs, encoding a wide range of TAAs for the activation of T-cell cytotoxicity against autologous tumor cells from tumors of different localization was examined.

The novelty of the present study, to the best of our knowledge, may be attributed to the demonstration the effectiveness of this approach by using a DNA construct which encodes epitopes of tumor-associated antigens of a group of oncological pathologies, not only limited to one nosology. In the present study, it was demonstrated that the possibility of using cell technology based on autologous DCs transfected with this DNA construct could effectively induce a cytotoxic antitumor immune response in the cell culture of patients with various oncological diseases [breast cancer (BC), colorectal cancer (CRC) and non-small cell lung cancer (NSCLC)]. This allows for the expansion of the scope of cellular immunotherapy based on antigen-primed DCs.

Materials and methods

Patients. Heparinized venous blood and tumor samples obtained between June, 2021 and March, 2022 from 9 patients with CRC, 13 patients with NSCLC and 18 patients with BC receiving treatment at the City Clinical Hospital No. 1 (Novosibirsk) and Novosibirsk Regional Oncology Center (Novosibirsk, Russia) were used in the present study (Table I). The inclusion criterion was the lack of history concerning surgery, chemotherapy and/or radiation therapy. Adenocarcinomas of the colon, lung or breast were histologically verified in all patients in accordance with the pathology. The presence of the HLA-A*02:01 allele was confirmed by genotyping DNA isolated from peripheral blood cells, using an ALLSET™ GOLD HLA A LOW RES SSP kit (54310D; Invitrogen; Thermo Fisher Scientific, Inc.). Voluntary written informed consent was obtained from all patients. All subjects gave their informed consent prior to their participation in the study. The study was conducted in accordance with the Declaration of Helsinki, and the study was approved by the local Research Institute of Fundamental and Clinical Immunology (RIFCI) Ethics Committee (No. 132 from June 4, 2021).

Polyepitope DNA constructs. Original DNA vaccine constructs were developed using the corresponding artificial genes based on the pmax plasmid (Addgene) (40). In particular, the following designs were applied: The pmax-CTL_1 construct containing epitopes from MAGE-A10, NY-ESO-1 and MUC-1; the pmax-CTL2 construct containing epitopes from MAGE-A3, PRAME, EpCAM and MUC-1; the pmax-CTL3 construct containing epitopes from EpCAM, CEA, GuanylylCyclase C and 5T4; the pmax-CTL4 construct containing epitopes from Legumain, VEGFR-1, VEGFR-2, FAP and Fos-related antigen-1; the pmax-CTL5 construct containing epitopes from Brachyury, SOX2, Snail1 and Snail2; the pmax-PolyTh construct containing epitopes from HER2, hTERT, p53, WT1, NY-ESO-1, VEGFR-2, survivin and MAGE-A3 (Table II). For DC transfection, a mixture of an equimolar amount of all DNA constructs was used, thereby providing a wide range of antigenic information.

Effective antigenic determinants recognized by cytotoxic T-lymphocytes were predicted using using NetMHC (41) and TEpredict software (42). Fragments containing epitopes capable of binding to the largest number of allomorphs of human MHC class II molecules (HLA-DR) were selected when designing the DNA constructs. The fact that amino acid residues flanking the epitope in the protein (the target antigen) can be important for interaction with the corresponding T-cell receptor was taken into account during fragment selection. The prediction of cytotoxic T-cell epitopes was carried out for the allelic variant of HLA-A*0201 class I molecules. Peptides for which the predicted pIC50 value (a characteristic of the affinity of the interaction of a peptide with an MHC molecule) was >6.8 were selected for further analysis.

Generation of mature DCs. Mononuclear cells (MNCs) were isolated from the peripheral blood of patients with CRC, BC and NSCLC using the standard method of Ficoll-Urografin density gradient centrifugation (ρ=1.077 g/ml) (43). The obtained MNCs were then incubated for 30 min in 5% CO_2 at 37˚C in order to isolate cells with increased adhesion ability. Non-adherent MNCs were isolated with the medium, precipitated by centrifugation at 415 x g for 10 min at room temperature, and cultured at 37˚C and 5% CO_2 in a 75 cm^2 culture flask (TPP Techno Plastic Products AG) to be further used at a concentration of $2x10^6$ cells/ml, in complete RPMI-1640 medium containing 10% fetal calf serum (FCS; HyClone; Cytiva), 2 mM L-glutamine (BioloT Ltd.), 10 mM HEPES buffer (MilliporeSigma), $5x10^{-4}$ M 2-mercaptoethanol (MilliporeSigma), 40 μg/ml gentamicin (Krka, d. d.) and 200 U/ml benzylpenicillin (OJSC Biosintez) prior to seeding. The adherent fraction was cultured in a 48-well plate (Greiner Bio One; Merck KGaA) at a concentration of $1x10^6$ cells/ml in 0.5 ml of complete RPMI-1640 medium, supplemented with 50 ng/ml recombinant human (rh) granulocyte-macrophage colony-stimulating factor and 100 ng/ml rhIL-4 (PeproTech, Inc.) for 96 h to obtain immature DCs. In order to obtain mature DCs, rhTNF-α (25 ng/ml) (PeproTech, Inc.) was added to the culture of immature DCs, and the cells were incubated for 2 days. In the resulting DCs, the expression of maturation and differentiation markers was assessed using flow cytometry (flow cytometer BD FACS Aria, BD FACS Diva v.6.0 software, BD Biosciences), using primary antibodies labeled with various fluorochromes [CD11c-PE-Cy7 (cat. no. 337216), CD83-APC (cat. no. 305312) and HLA-DR-FITC (cat. no. 327006)] (BioLegend, Inc.).

Magnetic transfection and evaluation of the efficiency of dendritic cell transfection. The magnetic transfection method was used as a method for delivering the DNA construct; unlike the electroporation method, it permits the acquisition of a cell culture with high viability (85.4±6.2%). The essence of

Table I. Clinical characteristics of the cancer patients in the present study[a].

Cancer type (n)	Colorectal cancer (n=9)	Non-small cell lung cancer (n=13)	Breast cancer (n=18)
Age (years), median (min; max)	45 (41; 55)	54 (43; 60)	45 (35; 58)
Sex			
Male	7	12	0
Female	2	1	18
Diseases stage, n			
Stage II	6	4	10
Stage III-IV	3	9	8
Localization (n)	Rectum (2) Sigmoid colon (5) Colon (2)	Peripheral Localization (13)	Ductal (18)

[a]All cancer samples were of adenocarcinoma origin (n=40).

magnetic transfection is the preliminary sorption of the DNA construct on the surface of magnetic beads, which penetrate into the cells in the culture of dendritic cells under the influence of a magnetic field. The magnetic transfection of mature DCs was performed in a total volume of 0.5 ml in a 48-well plate. Plasmid pmax (the control plasmid) and pmax-CTL1-8 (the target plasmid) were used for transfection. Magnetic transfection was performed using PromoKine reagents; the procedure was performed according to the manufacturer's protocol. Plasmids were dissolved in DMEM (State Research Center of Virology and Biotechnology 'Vector') in separate tubes; component was added in the ratio of 0.3 μg of DNA plasmid per 0.3 μl of MATra-A reagent, and the mixture was incubated at room temperature for 20 min. In parallel, DCs were precipitated by centrifugation at 266 x g for 5 min at room temperature, and RPMI-1640 medium was replaced with 250 μl of DMEM medium. Subsequently, the plasmid-MATra-A complex was added to the cells (25 μl per well); the plate was placed on a magnetic stand for 15 min. The medium was replaced after transfection: the DMEM was removed, and 300 μl complete RPMI-1640 medium were added. The transfected cells were incubated overnight in 5% CO_2 at 37˚C. The obtained dendritic cells were then plated with MNCs. The transfection efficiency was assessed using the Promo-Fluor-500 Nick Translation Labeling kit (PromoKine), with further analysis on a flow cytometer BD FACS Aria, BD FACS Diva v.6.0 software (BD Biosciences) using the Flow-Fish method (44,45).

Co-culture of DCs and MNCs. The obtained DCs transfected with pmax and pmax-CTL1-8 plasmids were co-cultured with the fraction of non-adherent MNCs (at a concentration of 1x10^6 cells/ml) for 120 h at 37˚C and 5% CO_2 to prime specific antigens (at a 1:10 DC to MNC ratio). Non-adherent MNCs cultured under the same conditions, as well as cells cultured in the presence of DCs not transfected with plasmids [MNCs+DC(0) group], were used as the controls.

Generation of autologous tumor cells. Tumor cells were obtained by cold trypsinization of the tumor samples obtained from the patients during the planned surgical intervention. A tumor sample was divided into small fragments in RPMI-1640 medium containing 80 μg/ml gentamicin, 400 U/ml benzylpenicillin and 5 μg/ml amphotericin B (PanEco Ltd.), placed in trypsin (Biolot Ltd.), minced with scissors, and left overnight at >4˚C. To inhibit trypsin, RPMI-1640 medium containing 10% FBS was added and mixed thoroughly; the homogeneous suspension was precipitated (185 x g, 10 min, room temperature), and the cell count was calculated in a Goryaev chamber (Minimed). The cells were frozen in FCS with 10% DMSO and stored at -70˚C. The cells were thawed and cultured in complete RPMI-1640 medium 24 h prior to the cytotoxicity assay. Additionally, the viability of the tumor cell culture was assessed by staining with erythrosin (0.5 mg/ml) (MilliporeSigma) at room temperature within 1 min, prior to the cytotoxicity assay, once the cell suspension has been mixed with the dye, which averaged 67.6±15.4%, and the settlement was calculated based on the number of living cells.

Evaluation of the cytotoxic activity of mononuclear cells against tumor cells. The cytotoxic activity was analyzed by assessing the level of lactate dehydrogenase (LDH) in a conditioned medium in the co-culture of the DC and MNC cell population and autologous tumor cells from patients with CRC, BC and NSCLC. The procedure was carried out according to the instructions of the manufacturer of the kit 'CytoTox96® Non-Radioactive Cytotoxicity Assay' (Promega Corporation). Following the co-culture of the non-adherent fraction of MNCs and DCs transfected with the plasmids, as well as the culture of the cells of the control groups for 120 h, the cell suspension was washed with RPMI-1640 culture medium (Biolot), and the resulting cells were seeded at a cell concentration of 1x10^6 cells/ml into round-bottom plates (TPP Techno Plastic Products AG; well volume, 50 μl) containing pre-thawed autologous tumor cells at a 10:1 ratio and incubated for 16-18 h at 37˚C and 5% CO_2. Cell seeding, and the experimental protocol were performed in accordance with the instructions for the 'CytoTox 96 Non-Radioactive Cytotoxicity Assay' kit (Promega Corporation). The optical density (OD) was measured on an Anthos 2020 spectrophotometer (Biochrom, Ltd.) at a single wavelength (490 nm). The cytotoxic effect was calculated according to the formula proposed by the kit manufacturer and expressed as a percentage: % cytotoxicity={[OD(experimental lysis)-OD(spontaneous lysis of effector cells)-OD(spontaneous lysis of tumor cells)]/[(OD(maximum lysis of tumor cells)-OD(spontaneous lysis of tumor cells)]} x100%. Thus, the formula considered the natural death of the tumor cells and MNCs.

Statistical analysis. Statistical data analysis was carried out using the GraphPad Prism software (GraphPad Software, Inc.). The normality of the sample distribution was assessed using the Kolmogorov-Smirnov test. The non-parametric Kruskal-Wallis test was used for statistical verification in the case of a non-normal distribution. An appropriate post hoc test was used after the Kruskal-Wallis test (e.g., Dunn's test). When comparing only 2 groups, a non-parametric Mann-Whitney

Table II. Amino acid sequences of the DNA constructs.

Construct name	Aminoacidic sequence of the DNA constructs
Poly-CTL-epitope construct 1	LMWITQCFLLLMWITQCFLGLYDGMEHLIAQIACSSPSVQLSLLMWITSLAQD APPLVLVCVLVALRLLEFYLAMGPGPGFLLLLLTVLSLSYTNPAVFLSFHISNLIL ILILSIVSLGLTYDGMLAASLLMWITQVSLLMWITQCFLFLWGPRAHASLLKFL AKVGLYDGMEHLSLLMWITQCALGSTAPPVAILILSIVFIAALLLLTVLTVSTAP PAHGVMLLVFGIDVYLFLWGPRAFLLLLLTVLTVVYLEYRQVPVLLLTVLTVV WITQCFLPVGPGPGA KFVAAWTLKAAA
Poly-CTL-epitope construct 2	VLVCVLVALLLLTVLTVVGLSNLTHVLLLLLTVLTVAKVAELVHFLALSRKVAE LALGSTAPPVAAAVVAGIVVLVKIWEELSVLALQSLLQHLVLDGLDVLLAAYL FLWGPRAFLLLLLTVLKVAELVHFLLSLSYTNPAVAAAYIFATCLGLYLEYRQV PVQLLALLPSLAYLGLSYDGLLAAYNLTHVLYPVFLWGPRALVRLRELLCELA ALYVDSLFFLAAYRTYWIIIELGPGPGFLLLLLTVLTVVILYENNVITIFLSFHIS NLSLLQHLIGLMLTDVSPEPLALVETSYVKVAAKMILKMVQLATLAKFSPYLS TAPPAHGVYLHARLRELCLGLSYDGLLGLKAGVIAVGPGPGAKFVAAWTL KAAA
Poly-CTL-epitope construct 3	LMIAVFTLVLYGPDTPIVLYGPDTPVHMADMVTWLYVCGIQNSVAAVVAGIVV LVAAYLWWVNNQSLFQMYLDTELFLTGNQLAVYLPRDVLAQLLLDLALWSLG AFEHLPSLKLDTMIFGVSLQTSYVFLGIVLALIGAIFLLVALLFGHMLKIGLKAG VIAVRTYWIIIELGIMIGVLVGVSLQTSYVFLAAYVFLGIVLAYLSGANLNLATV G IMIGVMISAGSFGLVLLTYDTHVAYMDTLIRRLILYENNVITIALIGAIFLLYL WWVNGQSLGQFRVYPELGLSAGATVGIYLNALEASVAAYIMIGVLVGVYLHSS KTEVLMMGNSAFAGPGPGAKFVAAWTLKAAA
Poly-CTL-epitope construct 4	YMISYAGMVAALQWMVQPHFLNLCEKPYPLYVYQNNIYLAALVCYGPGIAAY VLLWEIFSLGPGPGMVWKVAVFLATLHKQYHLVLLAASEAEVNLSDHTVAIGL FKCGIAVAATIFDRVYTIKQFCSTLTLKLLRGHTLVGLQREIEELSLTPFTPSLVK LWRYSYTAMQSKVLLAVGPGPGSLLAASEAEVAAVLLAVALWLAADLISYSFQ VSLTPFTPSLASSWEYYASVVLLWEIFSLILIHIGHHLTLNLTIMNVAGLLTCEAT VKMYRKMVFYIGLSPDRQFVAYSYTATYYIAFLYRDVTWIAAAMFFWLLLVA YQYGTTQTLFAVNWISYLFLQAETDKLAHLICYSFQVIMDPDEVPLYLGYPPPE IFIIDTTYPAAATLFWLLLTLMMYDDIAYSGPG PGAKFVAAWTLKAAA
Poly-CTL-epitope construct 5	SLPMLIWDSVAAYQNEEITALRMFPVLKVNVAAAYLYESYSMPVQLPNGLSPL ILSSGAYSPIAASMLPVSHNAALLSAVENELLLAAIPPPEIMLIWDSVLAAASQY PSLWSVRLIASWTPVAAYMNGSPTYSMAWLLPGTSTLGMALGSMGSVALQYR VDHLLVLAPQAQPIRVDHLLSAVAAYFLVKKHFNASMYLPGAEVMISMYLPG AGPGPGAKFVAAWTLKAAA
Poly-Th-epitope construct	MAAPGSARRPLLLLLLLLLLGLMHCASAPVIFSKAFSSLQLVFGSPYVSRLLGIC LLTDLQPYMRQFVAHLAKFVAAWTLKAAAHNQVRQVPLQRLRIVKVRRAIEQ LAAMDGFRLGFLHSGTAKSVLLPENNVLSPLRLGFLHSGTAKSVTCQARMFP NAPYLPSCLVLLKEFTVSGNILTIRLTAADHRQLEKRFVPDGNRISWDSISTFKN WPFLEGCACQFEELTLGEFLKLDRLGEFLKLDRERAKNKFNNFTVSFWLRVP KVSASHLE QYIKANSKFIGITELRKRSHAGYQTI

CTL, cytotoxic T-lymphocyte; Th, helper T-cell.

U test was used. A value of $P<0.05$ was considered to indicate a statistically significant difference. Data are presented as the median ± standard deviation. The number of individuals per group is indicated by 'n' in the figure legends.

Results

Generation of mature DCs and effector cells. The mature DCs of patients with CRC, BC and NSCLC with a >80% content of $CD11c^+HLA\text{-}DR^+$ cells (Fig. 1A) and a >40% content of $CD83^+$ cells (Fig. 1B) were obtained *in vitro* using the protocol described above. The unified protocol for DC generation was used for CRC-, BC- and NSCLC-derived cells, and it was observed to be effective for CRC- and BC-derived dendritic cell maturation. At the same time, in case of NSCLC-derived cells, the absence of a significant increase in the number of $CD83^+$ cells was demonstrated.

The described cell-based protocol allows the generation of mature DCs derived from peripheral blood monocytes of patients with CRC, BC and NSCLC. The resulting DCs are characterized by high expression levels of the $CD11c^+HLA\text{-}DR^+$ and $CD83^+$ markers (Fig. S1).

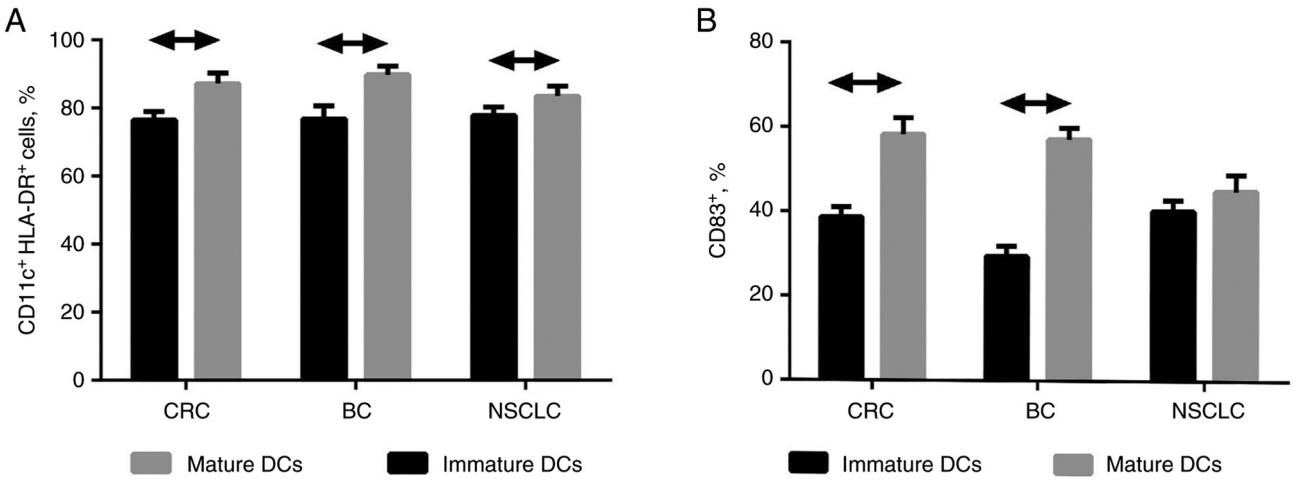

Figure 1. Relative count of (A) $CD11c^+HLA\text{-}DR^+$ cells in the monocyte-derived cell culture and of (B) $CD83^+$ cells in the $CD11c^+HLA\text{-}DR^+$ monocyte-derived cell culture from patients with colorectal cancer (n=9), breast cancer (n=18), and NSCLC (n=13); Immature DC-cell culture derived from patient monocytes by using cell culture with IL-4 and GM-CSF for 4 days; mature DC-cell culture derived from patient monocytes by using cell culture consistently with IL-4 and GM-CSF for 4 days and then with TNF-α for 2 days. The protocol for obtaining mature DCs included culturing with IL-4 (100 ng/ml) and GM-CSF (50 ng/ml) for 4 days, addition of a maturation stimulus TNF (25 ng/ml) followed by incubation for 2 days. Data are presented as the median ± standard deviation. Arrows indicate differences at $P<0.05$. HLA-DR, human MHC class II; NSCLC, non-small cell lung cancer; DC, dendritic cell; GM-CSF, Granulocyte-macrophage colony-stimulating factor.

The following stage involved the magnetic transfection of the obtained DCs with an original polyepitope DNA construct. An average efficiency of the DNA transfection of DCs was ~70%. An example of the evaluation of the efficiency of transfection using flow cytometry is presented in Fig. S2.

Following co-culture of the transfected DCs with the autologous MNCs obtained from cancer patients, final studies were conducted to evaluate the efficacy of the developed cell-based vaccine, which consisted of antigen-activated MNCs (effector cells) and mature DCs transfected with a polyepitope DNA construct. The content of $CD4^+$ and $CD8^+$ lymphocytes between groups of cancer patients did not exhibit significant differences and amounted to 52-62% ($CD4^+$ lymphocytes) and 24-27% ($CD8^+$ lymphocytes) (Table III). The efficiency of mounting a specific cytotoxic immune response in the co-culture of MNCs and DCs was evaluated using the cytotoxicity assay against autologous tumor cells.

Evaluation of the cytotoxic activity of MNCs against tumor cells. The LDH-releasing assay revealed that the cytotoxicity of the MNCs activated by DCs transfected with a polyepitopic DNA construct reached its values (45.5±7.1, 51.0±23.7 and 66.2±11.5% for patients with BC, NSCLC and CRC, respectively) when co-cultured with mature antigen-activated DCs transfected with the original DNA construct (Fig. 2). A significant increase in the cytotoxicity of cells in the culture of MNCs co-cultured with antigen-primed DCs, compared with groups of DCs and without transfection with the DNA construct of DCs, indicates the induction of an antigen-mediated cytotoxic immune response *in vitro*. These results directly demonstrate the effectiveness of the described approach.

Discussion

The emergence and development of the tumor development process is associated with a violation of the antitumor immune defense of the body (19,28,29). To effectively destroy tumor cells, there must be a sufficient number of effector cells that are able to recognize and destroy tumor cells. The aim of immune cell therapy is to obtain autologous antigen-primed dendritic cells capable of stimulating antitumor immunity. Hitherto, DNA constructs encoding epitopes of tumor-associated antigens have been successfully used previously (46). The following step in the development of this therapeutic approach is the creation of DNA constructs that can cover a wide range of tumor antigens, which is crucial, considering that the expression of tumor antigens may change qualitatively and quantitatively during chemotherapy and radiation therapy (47).

The use of a DNA construct encoding epitopes of TAAs of oncological diseases of general histogenesis will prevent tumor 'avoidance' when the antigenic composition of the effector cells changes. It may also permit the DNA construct to be used for the stimulation an antigen-specific anti-tumor immune response in oncological diseases that share a common histogenesis (e.g., BC, CRC and NSCLC). The cultivation and transfection of DCs *in vitro* makes it possible to overcome the tolerance of T-cells to tumor antigens and induces a complete cytotoxic immune response (48). In the present study, the ability of DCs, transfected with a DNA construct encoding epitopes of TAAs of neoplasms, to use a DNA construct, encoding epitopes of TAAs of various epithelial malignant neoplasms that have common antigens and are more specific for each specific pathology, was investigated. The use of transfection for the efficient delivery of antigens for the presentation of DC MNCs is a novel option for obtaining immune cells to stimulate antitumor immunity *in vitro*. HLA-A02:01-specific constructs were created and tested, the design of which was aimed at activating cytotoxic T-lymphocytes by presenting the corresponding TAAs on the surface of DCs. An analysis of cytotoxic activity revealed that the use of DCs transfected with an allele-specific DNA

Table III. Relative count of $CD3^+CD4^+$ and $CD3^+CD8^+$ cells in the co-culture of the transfected DCs with the autologous MNCs of patients with colorectal cancer, breast cancer and non-small cell lung cancer.

Cancer type (n)	Colorectal cancer (n=9)	Non-small cell lung cancer (n=13)	Breast cancer (n=18)
$CD3+CD4^+$, %	60.0±4.7	53.0±4.7	54.5±2.1
$CD3+CD8^+$, %	27.0±2.6	25.5±1.9	25.5±2.6

Data are presented as the median ± standard deviation. The content of $CD3^+CD4^+$ and $CD3^+CD8^+$ lymphocytes between groups did not exhibit significant differences. DCs, dendritic cells; MNC, mononuclear cells.

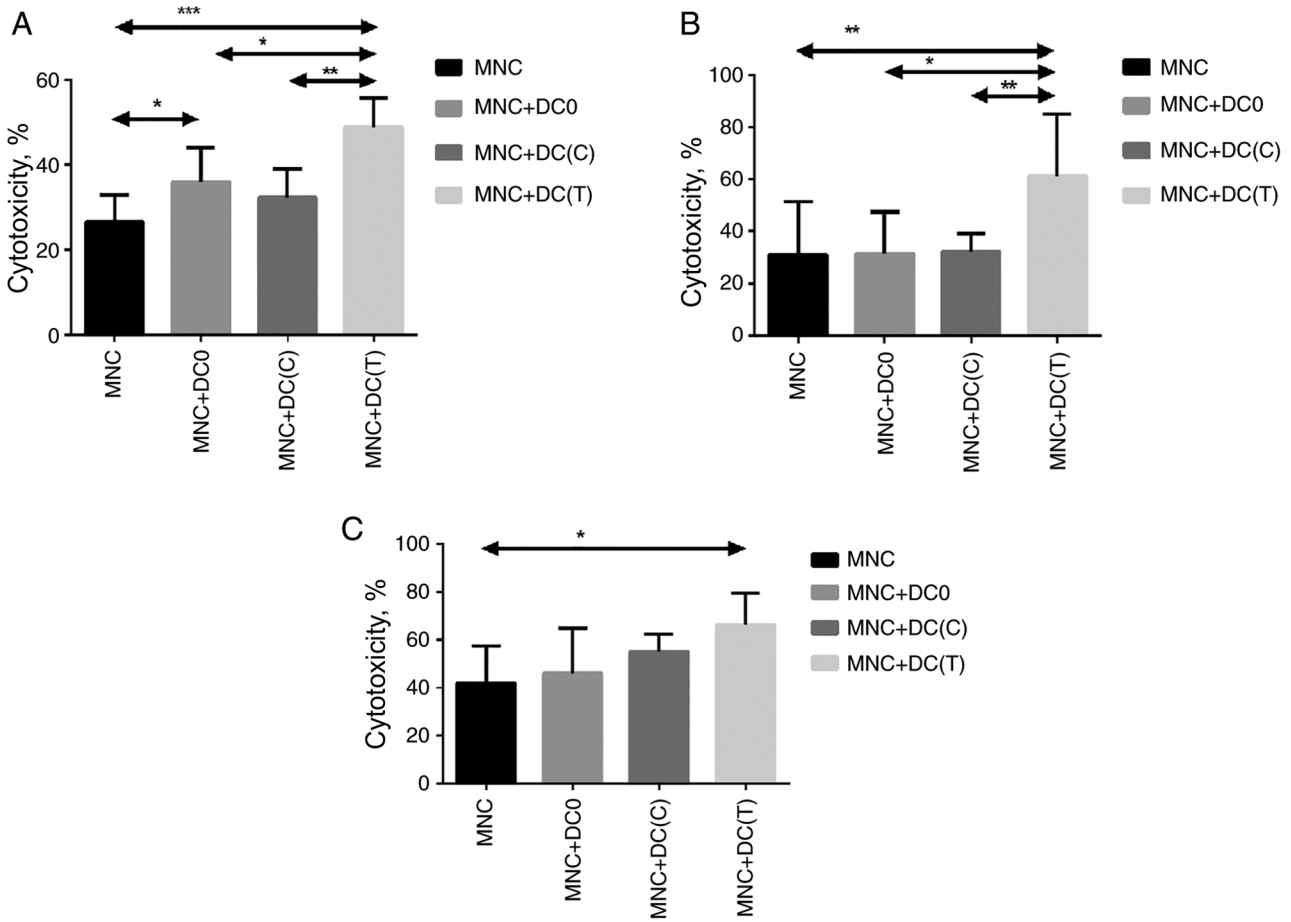

Figure 2. Cytotoxic response in a co-culture of MNCs from (A) breast cancer (n=18) from (B) NSCLC (n=13) and (C) colorectal cancer patients (n=9) with autologous DCs transfected with a DNA construct encoding the epitopes of tumor-associated antigens to autologous tumor cells (n=18). Data are presented as the median ± standard deviation. The arrows and lines indicate which experimental groups exhibited statistically significant differences. $^{***}P\leq0.001$, $^{**}P\leq0.01$ and $^{*}P\leq0.05$. MNCs, human mononuclear cells; NSCLC, non-small cell lung cancer; DC, dendritic cell; DCs(0), dendritic cells not transfected with a DNA construct; DCs(C), dendritic cells transfected with the control plasmid; DCs(T), dendritic cells transfected with a target plasmid (DNA construct).

construct encoding epitopes of TAAs of BC, CRC and NSCLC induced an antitumor immune response of MNCs against autologous tumor cells. It should be noted that when using immunotherapies based on the use of TAAs as a target, one should always be aware of safety issues, namely, the risks of developing unwanted side effects (damage to normal cells). Provided that TAAs are expressed on the surface of normal cells, there is also a possibility of autoimmune damage. The risk of developing such an undesirable effect increases with the use of immunotargeted drugs that are highly sensitive to one tumor-associated antigen (48-51). Cell therapy based on the use of DCs as key cells in the implementation of natural mechanisms for the induction and development of an antigen-specific cytotoxic immune response against tumor cells does not have a radical therapeutic effect, such as surgical, chemo- and radiation, immunotargeted therapy and, accordingly, does not have pronounced side-effects (31,52,53). The use of polyepitopic DNA constructs for the antigen priming of DCs renders it possible to induce a cytotoxic immune response against tumor cells that express a wide range of

TAAs, and of which the expression levels are much higher compared to those in normal cells. Thus, the use of DNA constructs encoding epitopes of the main TAAs for tumors of different localization, and also having similar antigenic characteristics for the antigen-priming of DCs, can effectively stimulate the antitumor cytotoxic immune response. This therapeutic approach renders it possible to address the treatment of tumors not only of different localization, and also to act on tumor cells which are characterized by antigenic escape during chemotherapy and radiation therapy.

Thus, due to the versatility of the composition of the DNA construct used, which contains the main most immunogenic epitopes of TAAs, new directions become available for the use of cell therapy based on mature DCs for the treatment of various epithelial malignant neoplasms.

In the present study, the use of immunogenic polyepitopes of TAAs as part of a DNA construct was successfully examined, considering also the most common antigenic profile of the studied tumors. It is known that the immunogenicity of a tumor is dependent on factors associated with the tumor microenvironment, including the functioning of antigen-presenting cells (DCs) and it is not determined by the tumor cell only (54). Key prerequisites for tumor immunogenicity include tumor antigenicity and the efficiency of antigen processing and presentation (55). The proposed approach to the production of mature antigen-primed DCs *in vitro* facilitates the solution of the problem of low antigen processing and presentation efficiency to a large extent. The use of a polyepitope DNA construct also facilitates covering for the most common tumor antigens. By contrast, the antigenicity of a tumor can vary, even within the same type of cancer. It has been demonstrated for a number of tumors (CRC, BC and NSCLC) that the immunogenicity of the tumor depends on the molecular profile of the antigens expressed by the tumor cells (56-59). Considering these differences in the expression of antigenic molecules, it is important to seriously consider this, since it is indicative of the intracellular mechanisms and patterns of development of various cancer subtypes. The development of cell therapy approaches in the future will be directed to the tumor cells of patients, considering the individual molecular profile of the tumor.

The present study bears two main limitations: In the framework of the study, the evaluation of changes in the morphological shape during the maturation of DCs was not photographed, and no *in vivo* experiments were conducted. The absence of images of mature DCs does not cancel the results of the evaluation of the expression of DC maturation markers using flow cytometry, which is a more objective and visual method for assessing the maturity of DCS. The study of the effectiveness of the developed approach under *in vivo* conditions is a logical continuation of the present study and justifies future research perspectives a separate publication.

In conclusion, the use of a DNA construct encoding epitopes of TAAs of various oncological diseases for priming autologous DCs of oncological patients enables the induction of a cytotoxic immune response in a culture of MNCs against autologous tumor cells of patients with breast cancer, colorectal cancer, and non-small cell lung cancer. This is of utmost importance for future practical application of polyepitope DNA constructs more universally, due to the greater representation of tumor antigens, towards cellular immunotherapy of various oncological diseases and the secondary prevention of relapse.

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Availability of data and materials

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Authors' contributions

The conceptualization of the present study was performed by SSe, AM and HS. Patient selection, clinical data collection, experimentation and statistical analysis were performed by VKu, EK, JS, JL, IO, JK, MK, AK, NK, SSi, AS, AV and VKo. Project administration was assigned to SSe and VKu. The acquisition of resources was made by AK, NK, VKo, SSi, AS and AV. SSe and HS supervised this study. Data validation was performed by EK, JS, JL, IO, AK, NK and JK. Figures and tables were prepared by VKu, JS, JK, EK, and IO. Writing and original draft preparation were performed by EK, VKu, SSi, AS, AV and MK. Additionally, SSe, MK and VKu performed writing, review and editing of the manuscript. The control of data collection and validation of the statistical analysis of the data was carried out by VKu. VKu and SSe confirm the authenticity of all the raw data. All authors have read and approved the final manuscript.

Ethics approval and consent to participate

All subjects provided their informed consent to inclusion prior to participation in the study. It was carried in accordance with the Declaration of Helsinki, and was approved by the ethics committee of the local Research Institute for Fundamental and Clinical Immunology (RIFCI) (No. 132 of June 4, 2021). Voluntary written informed consent was obtained from all patients.

Patient consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interests.

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