APPROACHES TO THE DEVELOPMENT OF THE DENDRITIC CELL AND NEOANTIGEN-BASED ANTITUMOR VACCINES

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Malignant neoplasms occupy a leading place among non-communicable diseases based on the number of patients and mortality rate. There are several fundamental approaches to cancer therapy, however, none of them are universal or show a high level of clinical response. Furthermore, all the approaches are characterized by a large number of adverse side effects. Today, immunotherapy used alone or in combination with other therapies is considered to be the most promising. Immunotherapy is usually the use of specific antibodies (immune checkpoint inhibitors) or special bioproducts, such as dendritic cells and artificially synthesized peptides, such as neoantigens. The review considers strategies for development of the dendritic cell- and neoantigen-based anticancer vaccines, the possibilities of their improvement and the efficacy of combining with other anticancer drugs. The summary of current trials of the dendritic cell- and neoantigen-based vaccines is provided along with a brief analysis of the basic strategies, achievements and challenges faced by the developers of such vaccines.

Keywords: dendritic cells, dendritic cell vaccine, neoantigens, neoantigen vaccines, anticancer therapy, targeted therapy, cell technology

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ПОДХОДЫ К РАЗРАБОТКЕ ДЕНДРИТНОКЛЕТОЧНЫХ И НЕОАНТИГЕННЫХ ПРОТИВООПУХОЛЕВЫХ ВАКЦИН

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Среди неинфекционных заболеваний одной из ведущих патологий по количеству пациентов и показателям летальности являются злокачественные новообразования (ЗНО). Для терапии ЗНО есть несколько принципиальных подходов, однако ни один из них не является универсальным и не обладает высоким уровнем клинического ответа. Кроме того, для всех подходов характерно большое количество нежелательных побочных явлений. Наиболее перспективным в настоящее время считают применение иммунотерапии — как самостоятельный подход либо в комбинировании с другими видами терапии. Иммунотерапия обычно представляет собой использование специфичных антител (ингибиторов иммунных контрольных точек) либо применение специальных биопродуктов, таких как дендритные клетки (ДК) и искусственно синтезированные пептиды, например, неоантигены (НА). В обзоре рассмотрены стратегии разработки противоопухолевых вакцин на основе ДК и НА, возможности их усовершенствования и эффективность комбинирования с другими противоопухолевыми препаратами. Представлена также сводка актуальных в настоящее время клинических испытаний ДК- и НА-вакцин с кратким анализом базовых стратегий, достижений и трудностей, с которыми сталкиваются разработчики данного вида вакцин.

Ключевые слова: дендритные клетки, дендритноклеточная вакцина, неоантигены, неоантигенные вакцины, терапия онкологических заболеваний, таргетная терапия, клеточные технологии

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Cancer is still one of major non-communicable cause of death in the adult population. According to the World Health Organization, cancer occupies the leading position based on mortality rate among people aged under 70 in 112 countries of the world [1]. The most common malignant neoplasms (MNs) by detection rate include breast cancer (BRCA), non-small cell and small cell lung cancer (NSCLC and SCLC, respectively), colorectal cancer (CRC), gastric cancer (GC), liver cancer (LC), prostate cancer (PC), cervical cancer (CC), thyroid cancer (TC), and bladder cancer (BLCA). Melanoma, various types of primary central nervous system cancers (neuroblastoma and glioblastoma) and oncohematological diseases can be considered as the most aggressive MNs. MNs with the highest mortality rate include lung cancer, CRC, LC, GC, BRCA, PC, CC, as well as esophageal cancer, pancreatic cancer, and leukemia [1]. MNs are found in people of various age and gender, different nationalities and professions. The important role in carcinogenesis is played by the genetic predisposition factors, harmful habits (such as tobacco smoking), and environmental factors(such as harsh industrial environment) that significantly increases the risk of MNs [2]. That is why early detection of MNs in the groups with occupational risks, adequate choice and implementation of timely anticancer therapy is important.

The main treatment for solid MNs (stages I–III) is surgical resection of the tumor with adjuvant and/or neoadjuvant therapy [3]. The combination therapy is often used: surgical treatment combined with radiation and chemoradiation therapy [3], as well as the combination with immunotherapy, for example, therapy with immune checkpoint inhibitors (ICIs) [3, 4]. In particular, in 2022 the U.S. Food and Drug Administration (FDA) has approved seven ICIs for the programmed cell death protein 1 (PD-1)/programmed cell death 1 ligand 1(PD-L1) pathway: pembrolizumab, nivolumab, durvalumab, atezolizumab, avelumab, cemiplimab, dostarlimab [4].

The other cancer immunotherapy option is represented by the use of the so-called dendritic cell vaccines (DC-vaccines) [5, 6]. It is believed that clinical efficacy of DC-vaccines is associated with targeting the populations of immunosuppressive

cells in the tumor microenvironment and subsequent immunogenic tumor cell death induction [7].

DCs are involved in antigen presentation, immune response regulation, inhibition of immunosuppressive T cells. DCs also can sensitize other effector cells of the innate antitumor immunity [5, 6]. Several DCs subpopulations are distinguished based on the origin and antigen receptors: myeloid DCs, lymphoid DCs, plasmacytoid DCs, Langerhans DCs, and monocyte-derived DCs [5, 6]. As a link of antitumor immune response, DCs are involved in recognition and presentation of the neoantigens, emerging de novo in the tumor cells, to the immunocompetent cells [5, 6]. It is rational to use this ability of the DCs loaded with tumor antigens ex vivo for further activation of the $CD4^+$ helper and $CD8^+$ cytotoxic T cells in order to determine the directions of the immune responses [8]. Today, only PROVENGE, the autologous cellular product, consisting of the antigen-presenting cells activated by the PA2024 recombinant chimeric protein, has been approved by FDA for treatment of PC based on the phase III clinical trial results () (NCT00779402).

Since the tumor neoantigens (NAs) stimulate specific antitumor immune response in the patient's body, the new personalized therapeutic approaches in the field of neoantigen vaccines (NA-vaccines) creation have been developed in recent years [9]. Neoantigens are highly specific for tumor cells. They can be divided into common ones, which are produced by the mutations in oncogenes and personalized ones (unique for the tumor found in a certain patient [10]. At least, two NA-based immunotherapy approaches are under activey development: peptide and RNA vaccines. Thus, peptide vaccines may contain the mixtures of synthetic peptides with adjuvants or the DCs loaded with peptides [11, 12].

The limitations of DC-vaccines are associated with time- and resource-consuming process of vaccine preparation. Sometimes this is the reason why the disease progression occurs, which reduces the clinical benefit of therapy. Furthermore, some patients might not survive to the end of the therapy course [5, 13]. The high cost of biological stimulators that are critical for correct DC differentiation and loading of DCs with antigens also prevents the timely production of vaccines and their introduction into clinical practice [5, 9, 13]. It is also pertinent to note that, despite the facts of achieving pathomorphological responses of tumors and stabilization of disease while administrating DC-vaccines, together with favourable pharmacological safety data, there is an objective problem of increasing the vaccine efficacy. This can be solved through various modifications of the existing vaccine compositions and combinations with other anticancer drugs [5, 6].

The aim of the review was to systematize the literature data in the approaches to the development of the DC- and NA-vaccines as candidate anticancer drugs in terms of optimizing methodological and some technological aspects of the drug development in order to overcome the abovementioned problem. The review also reports the features of interaction between the DC vaccines and human immune cells and the most advanced developments based on the data of preclinical and clinical trials (PCTs and CTs, respectively).

Clinical trials of the DC- and NA-vaccines for treatment of MNs

As of December 2022, a total of 410 and 96 records of the clinical trials (CTs) of the DC- and NA-vaccines, respectively, were found in the ClinicalTrials database [14]. Among all CTs focused on DC-vaccines, 191 CTs (46.58%) were completed, 45 CTs (10.97%) were terminated, 24 CTs (5.85%) were withdrawn (suspended). Among a hundred of active CTs, 32 CTs (7.80%) were assigned the status "active, not recruiting", 57 CTs (13.90%) had the "recruiting" status, and 11 CTs (2.68%) had the "not recruiting" status. The status of another 50 CTs (12.20%) was "unknown".

Among the successfully completed CTs of anticancer DC-vaccines, a total of 29 CTs (86% — phase II, 14% — phase III). were analyzed Table 1 provides basic information about the CTs conducted (title, phase, status, disorder, group of patients, DC-vaccine dosing regimen, drug in combinations, etc.). The CTs focused on clinical assessment of safety, tolerability efficacy of the DC-vaccines used in treatment of various cancer types have been distributed as follows. The group of malignant neoplasms (stage III) includes two CTs of DC-vaccines only for treatment of PC. The other two CTs are focused on DC-vaccines in combination with dasatinib for treatment of metastatic melanoma (stage III) or glioma in individuals receiving temozolomide (TMZ). The group of MNs (stage II) includes ten CTs of DC-vaccines used alone and 15 CTs of DC-vaccines used in combination with other pharmacotherapeutics, most often combinations with interleukin 2 (IL2), TMZ or interferon-α (IFNα). Other MNs are distributed as follows: glioma (five CTs), melanoma (three CTs), sarcoma (three CTs), prostate cancer (three CTs), ovarian cancer (two CTs) and breast cancer (two CTs). It must be acknowledged that the vast majority of clinical trials are focused on assessing the combination of DC-vaccines and ICIs. Information about the active CTs phases II and III is provided in Tables 2 and 3, respectively.

The number of CTs registered in the ClinicalTrials database and devoted to and NA-vaccines was about four times lower than that of the DC-vaccines. Among 96 CTs, 11 CTs were completed, eight were terminated, three were suspended; there were 60 active CTs and 14 CTs with unknown status. By analogy with DC-vaccines, clinical assessment of NA-vaccines involved mostly individuals receiving ICIs, and the spectrum of MNs targeted by CTs was almost the same. The safety and anticancer efficacy of the NA-vaccine in individuals receiving pembrolizumab and nivolumab were confirmed in NCT03633110 (phase II) only. Among eight terminated CTs, three were terminated due to long development time, and the other five were terminated due to underinvestment.

Analysis of DC-vaccines CTs (phase I and II) details has helped reveal a number of issues in this field. First, a small number of individuals (usually not exceeding 20) enrolled is the main factor of the CTs' termination. Second, complications with interpretation data obtained on different anticancer treatment regimens in the same CT. Third, specific design of the CT that includes a single cohort of patients or the CT without randomization. Despite the fact of achieving the endpoints of safety and tolerability of the anticancer vaccine, a common trend of moderate efficacy of the DC-and NA-vaccines administrated alone should be noted. It defines the relevance of their combination with other pharmacotherapeutics. However, there are exceptions. For example, the DC-vaccine for intratumoral administration obtained in the presence of IFNα and granulocyte macrophage colony-stimulating factor (GM-CSF) showed high immune responses even in the absence of tumor-associated antigen. It ensured complete regression of follicular lymphoma in some individuals who received low doses of rituximab [15]. It is important to note that the combinations of DC-vaccines with targeted or immunotherapy drugs showed higher efficacy than the DC-vaccines administrated alone. The objective response rate (ORR) reached 50%, and the difference in progression-free survival (PFS) and/or overall survival (OS)

Table 1. The main results of the completed clinical trials of DC-vaccines

Clinical trial (CT) title	Phase	Disorder	Number of groups	Dosing regimen	Drugs in combination	CT results	CT ID in ClinicalTrials.gov
Vaccine therapy in treating patients with metastatic prostate cancer that has not responded to hormone therapy	III	Prostate cancer	2	127 subjects. Experimental group: 3 infusions of Sipuleucel-T with an interval of two weeks. Control group: DC-vaccine, no PA2024 activation	No	Median OS in the experimental group was 25.9 months vs 21.4 in the placebo group. The 8-fold increase in the stimulated T cell counts relative to the controls was achieved in response to the DC vaccine (16.9 vs 1.99; $p < 0.001$)	NCT00005947
Provenge treatment and early cancer treatment (PROTECT)	III	Prostate cancer	2	176 subjects. Experimental group: 3 infusions of Sipuleucel-T with an interval of two weeks. Control group: DC-vaccine, no PA2024 activation	No	No differences in quality of life between the experimental and control groups were revealed. The 50th percentile of the PSA levels exceeding 3 ng/mL was 15 vs 12 months in the experimental and control groups	NCT00779402
Dendritic cell vaccines + dasatinib for metastatic melanoma	III	Metastatic melanoma	2	15 subjects. Intradermal injections of the drug (dose of 1×10^7 cells) in the vicinity of the lymph nodes on days 1 and 15 of the cycle. Cohort A — DC preparation + dasatinib (starting on day 1 of the cycle), cohort B — DC preparation + dasatinib (starting on day 1 of the second cycle — after 5 weeks)	Dasatinib	Among 13 CT participants, specific response of the T cells to the vaccine administration was achieved in 6. Partial response was achieved in 4 cases, and the disease stabilization in two cases. The other 7 participants did not respond to vaccination (disease progression). Cohort A vs cohort B: ORR 66.7% vs.28.6%, OS 15.45 vs 3.47 months and progression-free survival (PFS) 7.87 vs 1.97 months	NCT01876212
Study of a drug [DCVax®-L] to treat newly diagnosed GBM brain cancer (GBM)	III	Glioma	2	Control group (temozolomide + intradermal injections of DCVax-L). Experimental group (temozolomide + autologous PBMC (placebo). Injections (on weeks 0, 10, 20, 8, 16, 32, 48, 72, 96, and 120)	Temozolomide	The safety of use has been confirmed. The differences in the patients' survival between groups have not yet been revealed	NCT00045968
A study of ICT-107 immunotherapy in glioblastoma multiforme (GBM)	II–III	Glioma	2	124 subjects: 18–80 years. Group 1 (81) — therapy with autologous DCs, group 2 (43) — placebo	No	Median OS: DC-vaccine — 18 months, placebo — 16.7 months. Median PFS: DC-vaccine — 11.2 months, placebo — 9 months	NCT01280552
Dendritic cell vaccine study (DC/PC3) for prostate cancer	II	Prostate cancer	1	13 subjects. Subcutaneous injection of the DC-vaccine alone	No	Increased T cell proliferation in response to the DC-vaccine administration	NCT00345293
Vaccine therapy in treating patients with stage I, stage II, or stage III non-small cell lung cancer	II	NSCLC	1	32 subjects. Patients with histologically verified stage I-IIIB NSCLC. 16 intradermal injections, once a month	No	Assessment of immunogenicity: antigen-specific response to DC-vaccine is reported in 40%, non-specific response is reported in 40%	NCT00103116
Ovarian cancer vaccine for patients in remission	II	Ovarian cancer	3	63 subjects. 6–8 intradermal injections (forearm and thigh) (dose of 60×10^6 cells). Groups: control, randomization, no randomization	No	PFS 13 vs. 5 months and OS 42 vs 26 months in the cohorts DC-vaccine vs control, respectively	NCT01068509
Safety and effectiveness of a vaccine for prostate cancer that uses each patients' own immune cell	II	Prostate cancer	2	24 subjects. Subcutaneous injection of the vaccine. Cohort 1: placebo for 8 weeks, then DCs for more than 8 weeks. Cohort 2: DCs for more than 8 weeks	No	The DC-vaccine production method affected the efficiency of the T cell activation in response to the DC-vaccine administration	NCT00289341
Vaccine therapy in treating patients with liver or lung metastases from colorectal cancer	II	CRC	2	13 subjects. Cohort 1: Intradermal or subcutaneous injection of the DC-vaccine. Cohort 2: DC-vaccine + GM-CSF	No	There were little differences in the 2-year PFS between the cohorts (47% and 55%). There were no significant differences in the rate and intensity of the T cell immune responses between the cohorts	NCT00103142
Ovarian cancer vaccine for patients who have progressed during the CAN-003 study (CAN-003X)	II	Ovarian cancer	1	9 subjects. 3 doses of DCs were administered during 4 weeks, the other 3 doses during the subsequent 12 weeks, the remaining 6 doses during the subsequent 44 weeks	No	No data on efficacy available	NCT01617629
Vaccine for patients with newly diagnosed or recurrent low-grade glioma	II	Glioma	1	5 subjects. Administration of the drug on days 0, 14, 28	No	No data on efficacy available	NCT01635283
Therapy to treat Ewing's sarcoma, rhabdomyosarcoma or neuroblastoma	II	Sarcoma	2	44 subjects. Cohort A — baseline: administration of the CD25 and 8H9 depleted autologous lymphocytes + DC vaccine. Cohort B — baseline + recombinant IL7 (administration on days 0, 14, 28, 42)	No	The immune responses associated with the use of IL7 were reported in 57% of patients. The median OS was 2.4 and 4.3 months in the cohorts A and B, respectively	NCT00923351
A phase II feasibility study of adjuvant intra-nodal autologous dendritic cell vaccination for newly diagnosed glioblastoma multiforme	II	Glioma	1	11 subjects. Three doses of the vaccine were injected into the neck lymph node with an interval of two weeks	Temozolomide, radiation therapy	The $CD4^+$ cell activation was correlated to the patients' survival rate. The median PFS was 9.5 (5–41) months	NCT00323115
A pilot study of autologous t-cell transplantation with vaccine driven expansion of anti-tumor effectors after cytoreductive therapy in metastatic pediatric sarcomas	II	Sarcoma	1	42 subjects. Intramuscular injections of the DC-vaccine in a dose of 1×10^6 cells every 6 weeks	Indinavir (oral), infusions of IL2, IL7	The T cell responses were 60%, and the overall survival was two times higher in individuals who received DCs (73% vs 37%)	NCT00001566
DC vaccine combined with IL-2 and IFNα-2a in treating patients with mRCC	II	Metastatic kidney cancer	1	18 subjects. Induction therapy: Injections of the DC-vaccine into the lymph nodes — days 0 and 14 along with the IL2 (days 1–5 and 15–19) and interferon alpha (days 1, 3, 5, 15, 17, and 19) therapy. Adjuvant therapy: DC-vaccine (days 42, 70, and 98); IL2 — days 43–47, 71–75, and 99–103; IFNα (days 43, 45, 47, 71, 73, 75, 99, 101, and 103)	IL2, interferon alpha	Among 18 patients, the overall response was 50% with three complete responses. The counts of the circulating $CD4^+$ regulatory T cells were strongly correlated to the outcomes	NCT00085436
Vaccine therapy, tretinoin, and cyclophosphamide in treating patients with metastatic lung cancer	II	Lung cancer	1	24 subjects. Triple intradermal injection of the DC-vaccine every 14 days, the other three doses were injected once a month	Cyclophosphamide, tretinoin	The median OS was 8 months. The median PFS was 1.7 months. Among 14 patients, activation of the $CD8^+$ T cells associated with vaccination was achieved in 5 patients	NCT00601796
Vaccine therapy plus interleukin-2 in treating patients with stage III or stage IV melanoma	II	Melanoma	2	40 subjects. Cohort 1: DC-vaccine. Cohort 2: peptides injected in the form of emulsion with GM-CSF and the Montanide ISA-51 adjuvant.	IL2	In the cohort 1 the T cell immune responses were reported in 11–13%, while in the cohort 2 these were reported in 42–80%. ORR was observed in 10% of patients in the cohorts	NCT00003222

Table 1. Продолжение

External beam radiation with intratumoral injection of dendritic cells as neo-adjuvant treatment for sarcoma	II	Sarcoma	1	17 subjects. Intratumoral injections of three doses of the DC-vaccine (10^7 cells) during the course of radiation therapy.	Radiation therapy 50 Gy, 25 sessions	Survival of 67% of patients without systemic relapses within 2–8 years. In some cases, the immune response to the DC-vaccine administration was correlated to the clinical response	NCT00365872
Vaccine therapy, trastuzumab, and vinorelbine in treating patients with locally recurrent or metastatic breast cancer	II	BRCA	1	17 subjects. DCs + GM-CSF	Vinorelbine, trastuzumab	The increase in the share of the cytokine-producing $CD8^+$ cells by 36%	NCT00266110
Dendritic cell (DC)-based vaccines loaded with allogeneic prostate cell lines in combination with androgen ablation in patients with prostate cancer	II	PC	2	Cohort A. 3 months – androgen blockade, then 3 months — combination of androgen blockade + DC-vaccine. Cohort B: 3 months — combination of androgen blockade + DC-vaccine, then 3 months — androgen blockade	Androgen blockade	No data on efficacy available	NCT00970203
Dendritic cell/myeloma fusion vaccine for multiple myeloma	II	Multiple myeloma	3	203 subjects. Subcutaneous injection of the DC-vaccine (3×10^6 cells) in the upper third of the thigh on day 1 of each of 4 cycles of adjuvant therapy with lenalidomide	Lenalidomide, GM-CSF, melphalan	In the cohort with the корорте DC-vaccine + lenalidomide + GM-CSF (68 patients): 16% — complete response, 54% — partial response	NCT02728102
DC migration study for newly-diagnosed GBM (ELEVATE)	II	Glioma	3	64 subjects. Treatment course: 10 doses of the activated DC-vaccine (2×10^7 cells) were injected intradermally in the inguinal area	Temozolomide, basiliximab	The increase in the patients' median OS 16.5 vs 23.8 months, DC-vaccine with adjuvant (diphtheria toxoid) vs. DC-vaccine with no adjuvant. There were no significant changes in the PFS	NCT02366728
Study of gene modified immune cells in patients with advanced melanoma (F5)	II	Metastatic melanoma	1	14 subjects. After the chemotherapy course the patients received intradermal injections of 1×10^9 transgenic cytolytic T cells and 1×10^7 DCs, as well as IL-2 500,000 IU/m^2 twice a day for 14 days	IL2	No data on efficacy available	NCT00910650
A vaccine (CDX-1401) with or without a biologic drug (CDX-301) for the treatment of patients with stage IIB-IV melanoma	II	Melanoma	2	60 subjects. Experimental group: (CDX-301, CDX-1401, poly-ICLC). Control group: (CDX-1401, poly-ICLC)	Poly-ICLC, Flt3L, cytokine	In the experimental group stimulation of the immune response was reported in 53% of patients, while in the control group in was reported in 38% of patients. There were no significant changes in the time of recurrence (range 360–390 days)	NCT02129075
Vaccine therapy and 1-MT in treating patients with metastatic breast cancer	I–II	Metastatic BRCA	1	44 subjects. Intradermal injection of 6 doses of Ad.p53-DC on weeks 1, 3, 5 and 10, then every 3 weeks	1-methyl-D-tryptophan	Among 21 patients receiving the DC-vaccine, 1 complete response, 7 partial responses, and 2 cases of the disease stabilization were reported	NCT01042535
αDC1 vaccine + chemokine modulatory regimen (CKM) as adjuvant treatment of peritoneal surface malignancies	I–II	Mesothelioma	1	64 subjects. The DC-vaccine was injected in the lymph node once during the cycle in a dose of 3×10^6 cells + intradermal injection of the same dose.	Celecoxib, INFa-2b, rintatolimod	Average time to progression — 16 months, OS — 52 months. The treatment-associated chemokine production was reported	NCT02151448
Vaccination-dendritic cells with peptides for recurrent malignant gliomas	I–II	Glioma	1	22 subjects. DC-vaccine treatment regimen: initial injection in the lymph nodes (week 1), booster phase 1 (week 13) + poly-ICLC, booster phase 2 (week 33) + poly-ICLC.	Poly-ICLC	OS: dose of DCs (1×10^7 cells) + Poly-ICLC — (33 CI 14–37 months). Dose of DCs (3×10^7 cells) + Poly-ICLC — (13 CI 6–37 months)	NCT00766753

was up to 100% depending on the treatment regimen. Thus, DC-vaccines in combinations with other therapy may have a more prominent anticancer effect ensuring higher OS.

The other trend found is — DC- and NA- vaccines are considered as a "last choice therapy" option. It may be the cause of their low efficacy in the CTs in a group of individuals with late-stage cancers. Alternatively, stimulation of the tumor-infiltrating immune cells and local immune responses has all the chance to demonstrate much better efficacy for treatment of early-stage cancers, when it is necessary to prevent metastasis.

Optimization of some manufacture and application steps of biotherapeutic anticancer vaccines

Options of accelerating, simplifying and cost-reducing of the DC-vaccines manufacturing

1. Options for accelerating the DC-vaccines manufacture process

The use of nucleic acids to load the dendritic cells is the first approach to accelerating the DC-vaccine manufacture [9]. Synthesis of nucleic acids is a less time-consuming process than the synthesis of target peptides. Similarly, the nucleic acid purification procedure is less time-consuming than purification of the peptides or polypeptides. Nucleic acids, that are more stable than peptides, are adjuvants that can activate pro-inflammatory molecular pathways involving the Toll-like receptors (TLR) associated with activation of innate immunity [16].

The second approach involves modification of cultivating conditions of manufacturing cell strains. For example, the transfer of murine bone marrow progenitor cells into monolayers of murine OP9 stromal cells expressing the delta-like Notch 1 ligand (OP9-DL1) after three days of incubation with the FMS-like tyrosine kinase 3 ligand (FLT3L) led to the fact that the cells expressed the murine markers (CD103, CD24, DEC205 and CD8α) of myeloid DCs, the population that did not arise after incubation with FLT3L only. The transcriptional gene expression profile of such DCs was most similar to that of autologous DCs of the spleen. Meanwhile, the survival rate of laboratory animals increased, which could be due to enhanced lymphocyte migration to the tumor lesions [6]. The co-culture of human hematopoietic stem and progenitor cells and OP9-DL1 enabled a 20-fold increase in the yield of DCs of all types relative to conventional cell culture methods [17].

The third approach involves stimulation of the cell culture with various cytokines, such as GM-CSF [17, 18]. The transcriptional profiles of the DCs obtained were almost identical to that of primary DCs, while the cells themselves demonstrated normal cytokine responses to TLR agonists, including secretion of IL12, TNFα and IFNγ, and effectively induced the $CD4^+$ and $CD8^+$ T cell proliferation [17, 18].

The fourth approach was implemented by using the genetic editing technologies. Thus, viral transduction [19] and RNA interference methods [20] together with the CRISPR/CRISPR-Cas9 genome editing system [21] were used to generate the DC-vaccines. Pre-clinical trials showed that all methods were highly effective and could presumably be scaled to the DC-vaccines manufacture.

Another reported vector-free approach for acceleration of the DC-vaccine preparation is based on the Cell Squeeze® technology which involves forcing the target molecules through

Table 2. Open-label clinical trials of the DC- and NA-vaccine efficacy (active, not recruiting)

Vaccine title	Vaccine composition	Phase	Disorder	Patient recruitment	Vaccine dosing regimen	Drugs in combination	CT ID in ClinicalTrials.gov
no	DCs + RNA	III	Uveal melanoma	200 individuals, (18–75), M and F	Group A — 8 vaccine doses within 2 years, group B — control	no	NCT01983748
ADCTA-SSI-G1	DCs + tumor cells	III	Glioblastoma multiforme	118 individuals (18–70), M and F	10 doses: 2–4 × 10^7 cells for the first dose (double dose) and 1–2 × 10^7 cells for the doses 2–10, 3 vaccines twice a week	no	NCT04277221
DEN-STEM	DCs + mRNA of cancer stem cells, surviving or hTERT	III	Glioblastoma	60 individuals, (18–70), M and F	Intradermal injection of DCs, up to 6 cycles of temozolomide after 4 weeks	Adjuvant temozolomide	NCT03548571
GIMI-IRB-19006	DCs	II	Solid cancer types	100 individuals, (18–80), M and F	No details available	no	NCT04085159
CCRG12-001	DCs	II	Acute myeloid leukemia	130 individuals, (18+), M and F	Vaccination with DCs, combining with chemotherapy is possible (if earlier prescribed)	no	NCT01686334
no	DCs	II	Acute myeloid leukemia	75 individuals, (18+), M and F	No details available	no	NCT03059485
ADCV01	DCs	II	Glioblastoma	24 individuals, (20–75), M and F	A total of 10 doses (1 mL/dose; 2 ± 0.5 × 10^7 cells/dose) of ADCV01 will be administered to patients in the experimental group. ADCV01 will be injected in the axillary subcutaneous regional lymph nodes on both sides (half of the volume about 0.5 mL ADCV01) once a week for the first 4 doses; the next 2 procedures will be performed every two weeks. The last 4 procedures will be performed every 4 weeks	no	NCT04115761
no	DCs with tumor lysate (with a concentration of 1x106 cells)/ or WT1 and MUC1 proteins (for patients with certain HLA type (HLA-A2)) + immature DCs (as a load with the carrier protein - keyhole limpet hemocyanin (KLH))	II	Ovarian cancer	36 individuals, (18+), F	Three injections in the inguinal area with an interval of two weeks (6 weeks)	no	NCT00703105
DENDR1	DCs + tumor lysate	II	Glioblastoma	76 individuals, (18–70), M and F	4 vaccines every second week (vaccines I, II, III, IV), another 2 vaccines monthly (vaccines V, VI) and the last vaccine (vaccines VII) 2 months after the sixth one. Injections I, V, VI and VII will deliver 10 million DCs + tumor lysate, while the other injections will deliver 5 million cells only	no	NCT04801147
IRST153.04	DCs + tumor homogenate	II	Metastatic CRC	19 individuals, (19+), M and F	Each vaccine dose contains 1 × 10^7 DCs + tumor homogenate.	no	NCT02919644
IRST100.42	DCs + tumor homogenate	II	Head and neck cancers, neuroendocrine tumors, soft tissue sarcoma	51 individuals, (18+), M and F	7–14 × 10^6 DCs + tumor homogenate, delivered by intradermal injection (day 1)	no	NCT04166006
HER2 DC1	HER2-sensitized DCs	II	BRCA, HER2+ BRCA	60 individuals, (18+), F	Ultrasound-guided intranodal injections, each dose containing 1.0–2.0 × 10^7 cells will be injected in one left and one right inguinal lymph nodes	no	NCT03630809
CSTI571ADE60	DCs + peptides of bcr/abl, WT-1 + proteinase-3	II	Chronic myeloid leukemia	30 individuals, 18–80, M and F	Ten vaccinations within 26 with the use of the 10 × 10^6 freshly thawed DCs, intradermal injections (1–2 mL)	no	NCT02543749
IOR-IISML42037	DCs	II	SCLC	20 individuals, (18+), M and F	Intradermal injections (no more than 6 doses) on weeks 1, 3, 6, 9, 21, 33	Atezolizumab, carboplatin	NCT04487756
DC1	DCs	II	BRCA (stages I–III), HER2+ BRCA	110 individuals, (18+), F	Weekly intranodal injections between weeks 1 and 6 (the window between the vaccines 8–21). The booster vaccines will be administered with an interval of about 3 months on months 6, 9 and 12 (with an interval of +/– 1 month)	WOKVAC vaccine	NCT03384914
MSDCV	DCs	II	Hepatocellular carcinoma	600 individuals, (18–70), M and F	Once every 4 weeks during 0–20 weeks, about 5 × 10^7 cells per dose, a total of 6 intravenous injections	Cyclophosphamide (Endoxan)	NCT04317248
MC1685	DCs	II	Lymphoma	44 individuals, (18+), M and F	Therapy with DCs on days 2, 8 and 15 of the cycles 2 and 3, day 2 of the cycles 4 and 5	Pembrolizumab, 13-valent pneumococcal conjugate vaccine	NCT03035331
CA209-7R9	DCs + NA	II	Hepatocellular carcinoma, CRC with liver metastasis	60 individuals, (21+), M and F	10 doses of vaccine will be administrated by intradermal route together with the nivolumab AT	Nivolumab (Opdivo)	NCT04912765
IRST172.02	DCs + tumor lysate/homogenate	II	Stages III–IV melanoma	24 individuals, (18–70), M and F	Intradermal injections of the vaccine on weeks 1, 4, 6 and 8 during the induction phase and every four weeks during the maintenance phase, up to 14 vaccine doses (each dose is followed by administration of 3 MU of IL2 per day)	IFNα	NCT01973322
CCRG13-002	DCs + WT1 mRNA	II	Malignant pleural mesothelioma	20 individuals, (18+), M and F	4 intradermal injections of 8–10 × 10^6 DCs + WT1 mRNA; on day 14 +/- 3 days after the start of each chemotherapy cycle	Platinum-based drugs/ pemetrexed	NCT02649829
no	DCs + A2B5+ stem cells	II	Glioma, glioblastoma multiforme	100 individuals, (18–70), M and F	8–10 × 10^6 DCs in 0.5 mL of phosphate buffer saline are administered by intradermal injection in the shoulder close to the posterior surface of the neck to facilitate the DC transfer into the neck lymph nodes	Temozolomide	NCT01567202
MG-7-DC	DCs + MG-7 antigen	II	GC	45 individuals, (18–80), M and F	Six intranodal injections of the DC vaccine will bedone on days 1, 8, 15, 21, 28, 35; 1–3 × 10^6 cells	Sintilimab	NCT04567069
CCRG14-001	DCs + WT1 mRNA	II	Glioblastoma multiforme	20 individuals, (18+), M and F	Weekly (+/- 1 day) injections of DCs + WT1 mRNA during 3 weeks	Temozolomide	NCT02649582
GlioVax	DCs + tumor lysate	II	Glioblastoma	136 individuals, (18+), M and F	Vaccination with DCs + tumor lysate (7x, 2–10 × 10^6 DCs per intradermal injection, weekly on weeks 11–14, then on weeks 17, 21, 25)	Temozolomide	NCT03395587

Table 2. Продолжение

no	DCs + IL12	II	Glioblastoma	10 individuals, (18–75), M and F	Intradermal injection in the vicinity of the neck lymph node after surgery with subsequent radiation therapy (2 Gy/day for 30 days).	Temozolomide	NCT04388033
pp65 DC	DCs + pp65-shLAMP mRNA + GM-CSF	II	Glioma, glioblastoma multiforme	175 individuals, (18+), M and F	Intradermal injection on day 22–24 after the first course of temozolomide, then with an interval of 2 weeks. The doses 4–10 will be administered on day 22–24 of each cycle of temozolomide. Administration of the doses will be resumed until the total number reaches 10 or the disease progression/unacceptable toxicity is reported	Tetanus-diphtheria toxoid	NCT02465268
PDC*lung01	DCs + synthetic peptide (NY-ESO-1, MAGE-A3, MAGEA4, Multi-MAGE, SURVIVN, MUC1) or + peptide obtained from the Melan-A antigen	II	NSCLC	64 individuals, (18+), M and F	In the cohorts A1 (low dose cohort) and A2 (high dose cohort), patients with NSCLC will be treated with low/high doses of PDC*lung01, administered by serial subcutaneous injections and then by intravenous route. In the cohorts B1 and B2, the first injection of PDC*lung01 will be started within 48 h after the first anti-PD-1 infusion. The fourth PDC*lung01 injection will be started within 48 h after the infusion of the second anti-PD-1 cycle	Alimta, Keytruda	NCT03970746
no	Flt3L/CDX-301 + Poly-ICLC	II	Non-Hodgkin lymphoma, metastatic BRCA, squamous cell carcinoma of the head and neck	56 individuals, (18+), M and F	Intravenous infusion of 200 mg of pembrolizumab (Keytruda) for 30 min, then DCs together with Flt3L	Keytruda, hiltonol	NCT03789097
no	DCs + tumor lysate	II	Pediatric glioblastoma	25 individuals, (3–21), M and F	4 weekly intradermal injections of DCs + tumor lysate, with 3 subsequent monthly booster vaccines containing the tumor lysate and additional booster vaccines every three months	Cyclophosphamide (Endoxan), nivolumab, ipilimumab	NCT03879512
Pro00082570	DCs + CMV pp65-LAMP mRNA	II	Glioblastoma	112 individuals, (18+), M and F	2×10^7 DCs are administered by intradermal route in the inguinal area on both sides (the dose is split evenly between two sides of the inguinal region). The patients will receive a total of up to 10 doses of the DC-vaccine	Temozolomide, tetanus-diphtheria toxoid, varlilumab	NCT03688178
no	DCs + WT1 mRNA	II	High grade glioma, diffuse intrinsic pontine glioma	10 individuals, (1–17), M and F	1) Induction immunotherapy: intradermal injection of DCs + WT1 mRNA, weekly (–1 day, +2 days) during 3 weeks, starting from week ≥ 1 after radiation therapy. 2) Induction immunotherapy: intradermal injection of DCs + WT1 mRNA, weekly (–1 day, +2 days) during 3 weeks, starting from week ≥ 4 after apheresis	Temozolomide	NCT04911621
no	DCs +GSC-DCV	II	Glioblastoma	40 individuals, (18–70), M and F	Every 3 weeks if there is no disease progression or unacceptable toxicity	Camrelizumab	NCT04888611
GCO 13-1347	Flt3L+Poly-ICLC	II	Low-grade B-cell lymphoma	21 individuals (18+), M and F	Intratumor injections on days 1–5 and 8–11. Weekly intratumor injections of Poly-ICLC on weeks 2–8	Hiltonol	NCT01976585

the membrane pores emerging due to temporary membrane integrity disruption [22]. It has been shown that this DC loading technique can be used *ex vivo* and it is suitable for transfer of various antigens to cytosol [23].

2. Options for reducing the cost of the DC-vaccines manufacture process

Among all available options for reducing the cost of DC-vaccines there are exosome preparations obtained from DCs (DEXs). DEXs are considered as more technologically feasible and less expensive compared to conventional DC-vaccine preparation. Both *in vitro* and *in vivo* studies have shown that DEXs can activate the $CD4^+$ and $CD8^+$ T cells and stimulate the effective antigen-specific responses of cytotoxic lymphocytes. However, the desired anticancer efficacy has not been achieved in several CTs, putting into question the prospects of DEXs application [24]. The DCs pretreatment with interferon — (IFNγ) resulting in the increased expression of *CD40*, *CD80*, *CD86* and *CD54* is an option to increase the DEX efficacy. However, this approach, well proven in PCTs [25], was less effective in the CT (phase II) [26].

3. Options for simplifying the DC-vaccines manufacture process

Preparation of the DC-vaccines based on primary DCs extracted from the patient's peripheral blood is much simpler than ex vivo DC preparation, with such limitation as the low DC content (less than 1%) in the monocyte fraction [27]. Low circulating DCs counts have been revealed in blood samples of patients with melanoma [28] and breast cancer [29], while abnormal DC differentiation is reported in the breast cancer and pancreatic cancer models [30]. Therefore, the effectiveness of DCs isolation from the peripheral blood of patients with these tumor types was minimal. Since the successful implementation of this approach has yet been demonstrated only *in vivo* in the murine model with xenotransplantation of B16/F10 and B16-Flt3L cells (melanoma) as well asMC38 cells (CRC) [31], the prospects of preparation the DC-vaccines (DCs type I) against the majority of tumors seem to be hardly feasible.

Options of the anticancer vaccines application in combination therapy

Growth factors

The combinations of DC-vaccines and growth factors are designed to enhance the antigen-specific response. GM-CSF is most often used in combinations with DC-vaccines because it functions as a hematopoietic growth factor and immunomodulator. GM-CSF was also used as a low-toxic adjuvant during treatment with the DC- or NA-vaccines containing peptides [32]. Another approach based on the use of DC-vaccines and FLT3L has been reported. Thus, a significant increase in the generation of autologous DCs, including plasmacytoid DCs, has been revealed in the murine models in the presence of FLT3L. It is assumed that the increase in the mature DCs functional activity in the presence of FLT3L is mediated through the signaling pathways involving phosphoinositide 3-kinase (PI3K) and mTOR kinase [33].

ICIs

The combinations of ICIs and DC-vaccines lead to activation of T cells and NK cells, reduced immunosuppressive activity

Table 3. Open-label clinical trials of the DC-vaccine efficacy (recruiting)

Vaccine name	Vaccine composition	CT phase	Disorder	Patients	Vaccine dosing regimen	Drugs in combination	CT ID in ClinicalTrials.gov
NL55823.000.15	DCs + NA	III	Melanoma	210 individuals, (18+), M and F	No more than 3 cycles, 3 intranodal DC injections ($3–8 \times 10^6$) per cycle.	no	NCT02993315
DCP-001	DCs	II	Acute myeloid leukemia	20 individuals, (18+), M and F	Low dose — patients receiving 4 2-week 25×10^6 cells vaccines/vaccination with DCP-001 and 2 revaccinations with 10×10^6 cells/vaccination, High dose — patients receiving 4 2-week 50×10^6 cell vaccines/vaccination with DCP-001 and 2 revaccinations with 10×10^6 cells/vaccination	no	NCT03697707
no	DCs	II	Acute myeloid leukemia	63 individuals, (18+), M and F	2–3 vaccine doses with an interval of 4 weeks	no	NCT01096602
DC-005	DCs + mRNA of tumor cells, survivin or hTERT	II	Prostate cancer	30 individuals, (18–75), M	No details available	no	NCT01197625
no	DCs + TARP peptide	II	Prostate cancer	40 individuals in 2015 (actually 14 in 2020), (18+), M	20×10^6 of viable cells/dose were administered intradermally on weeks 3, 6, 9, 12, 15 and 24	no	NCT02362464
no	DCs + total tumor RNA (ttRNA)	II	Medulloblastoma	26 individuals, under 30 (children and adults), M and F	Intradermal injection of 1×10^7 cells every 2 weeks, a total of 3 doses	no	NCT01326104
AV-GBM-1	DCs + tumor-associated antigens (AV-GBM-1)	II	Glioblastoma	55 individuals, (18–70), M and F	No details available	no	NCT03400917
no	DCs + GM-CSF	II	Kidney cancer	38 individuals, (18+), M and F	3 vaccines with an interval of 3 weeks	no	NCT00458536
no	DCs + NA	II	CRC	25 individuals, (18–75), M and F	No details available	no	NCT01885702
TLPLDC	DCs + yeast cell wall particles + tumor lysate	II	Melanoma	184 individuals, (18–99), M and F	6 flasks containing a single dose for intradermal injection x 3 every months with further booster injections after 6, 12 and 18 months in the same area of the lymph node drainage (preferably in the anterior thigh)	no	NCT02301611
no	DCs + WT1 mRNA	II	Acute myeloid leukemia	5 individuals, (18–70), M and F	4 doses, once every 2 weeks	no	NCT03083054
no	DCs + GM-CSF	II	Ovarian cancer, primary peritoneal cancer, fallopian tube cancer	23 individuals, (18+), Ж	Subcutaneous injection once every 3 weeks	Imiquimod	NCT00799110
no	DCs + NY-ESO-1 protein	II	MNs without clarification	6 individuals, (16+), M and F	The patients can receive 3 additional doses of the peptide vaccine based on the NY-ESO-1 dendritic cells (157–165) after day 90 of therapy	Fludarabine phosphate, cyclophosphamide	NCT01697527
no	DCs + tumor lysate	II	Gliomas, glioblastoma	60 individuals, (18–70), M and F	Intradermal injection of DC-vaccine and tumor lysate (in all patients). Cohort 1 — optional application of the placebo cream, the vaccine is supplemented by saline, cohort 2 — the vaccine is supplemented by resiquimod, cohort 3 — the vaccine is supplemented by hiltonol	Resiquimod, hiltonol	NCT01204684
Ad.p53-DC	DCs + p53	II	SCLC	14 individuals, (18+), M and F	4 cycles of 21 days: the individuals will receive a p53-vaccine on days 1 and 15 of cycle 1, then once again on day 8 of cycle 2. Adjuvant immunotherapy started on day 1 of cycle 5: three additional doses of the p53-vaccine (every 4 weeks during 12 weeks)	Nivolumab, ipilimumab	NCT03406715
no	DCs + CT-011	II	Multiple myeloma	35 individuals, (18+), M and F	The DC vaccination is performed 1–3 months after the autologous transplantation. Vaccination is performed with an interval of 6 weeks	CT-011	NCT01067287
no	DCs + cytokines	II	Breast cancer	400 individuals, (18–75), M	4 cycles of the DC-CIK treatment (annually)	Capecitabine	NCT02491697
no	Exact formulation is not available	II	Prostate cancer	19 individuals, (18+), M	Intradermal injection 6 times every 2 weeks, then 9 times every 4 weeks	Nivolumab	NCT03600350
BVAC-C	Autologous B cells and monocytes trasfected with the HPV gene E6E7	II	Cervical MNs	32 individuals, (20+), F	Intravenous injections of BVAC-C on weeks 0, 4, 8, then on weeks 0, 4, 8, 12. After that in combination with topotecan on weeks 0, 4, 8, 12	Topotecan	NCT02866006
no	DCs + IL2	II	Melanoma	1230 individuals, (12+), M and F	1×10^7 to 2.5x108 DCs with the MART-1 peptide administered intravenously for 20–30 min, about 4 h after the T cell administration	Fludarabine phosphate, cyclophosphamide, IL2	NCT00338377
no	DCs + tumor proteins	II	Melanoma (stage III–IV)	7 individuals, (18+), M and F	The patients are administered mature DCs on day 1 or 2 of the course 2 or 3 after the low temperature exposure	Pembrolizumab	NCT03325101
no	DCs + NY-ESO-1 and Melan-A/MART-1 peptides	II	Melanoma	36 individuals, (18+), M and F	Intradermal administration of 100 μg/L of the peptide (NY-ESO-1 and Melan-A/MART-1) + 10 to 15×10^6 DCs per pertide antigen (NY-ESO-1 and Melan-A/MART-1) (no more than 50×10^6 cells in total)	Hiltonol, montanide	NCT02334735

of regulatory T cells [5, 34], and therefore to the increase in the DC- vaccine efficacy. In turn, the DC-mediated activation of NK cells and DC γδ T cells [35, 36] can increase the efficacy of ICIs. Synergistic antitumor effect of the combination of nivolumab and DC-vaccine was revealed in individuals with BRCA, myeloma, melanoma, lung cancer, lymphoma and glioblastoma [37]. In addition, the DC-vaccine was proven to be safe for patients; low number of side effects related to the use of nivolumab was reported [37].

NK cells

One more promising approach involves the combination of anti-cancer DC-vaccines and NK cell-based vaccines. NK cells present in the tumor microenvironment can produce a number of chemokines that positively affect the DC activity along with the FLT3L that enhances the autologous DC generation [38]. Furthermore, the activated NK cells can kill immature DCs and induce the adaptive immune response in the secondary

lymphoid organs. The mature DCs produce cytokines (mainly IL2, IL12, IL18) that stimulate production of IFNγ, TNFα or GM-CSF by the NK cells, thereby accelerating the DC maturation process [39].

Modifications of DC- and NA-vaccines

DC-vaccines

The contemporary trend in the development of anti-cancer vaccines is represented by the targeted approach based on the tumor-associated antigens (TAAs). These include overexpressed antigens, normal differentiation antigens and cancer stem cell antigens, as well as NAs. A peptide, chimeric protein, DNA or RNA can be the active ingredient of such vaccines [16].

One approach to modification of DC-vaccines involves the use of nanoparticles that are easily internalized by DCs through endocytosis and can be used as carriers of nucleic acids or peptides [32]. In this context, nanoparticles have some advantages: immunogenicity and the ability to be translocated through lymphatic vessels, if the particle size does not exceed 200 nm. The tumor antigens can be conjugated with nanoparticles by adsorption, encapsulation, chemical conjugation and self-assembly [32].

Another promising approach to modification of DC-vaccines involves genetic reprogramming of somatic cells by inducing the expression of key cell differentiation factors. The moDCs are more appropriate for this approach compared to other DCs. For example, the SmartDC technology enables reprogramming of autologous $CD14^+$ monocytes using the lentiviral vector that carries genes encoding GM-CSF, IL4 and TRP2 (dopachrom tautomerase). Transduction with the viral vector triggers differentiation of monocytes into the $TRP2^+$ moDCs.The SmartDC technology is simpler and less time-consuming compared to conventional DC-vaccine preparation [19].

NA vaccines

Developments of machine learning algorithms and neural networks allow for rather accurate identification of the patient's NAs and predicting the protein (peptide) structure [9]. Information about the predicted and tumor NAs is systemized in the specialized databases, such as dbPepNeo [40]. However, not all tumor NAs can be used to develop the NA-vaccines. Such parameters of NAs, as allogeneity, clonal distribution, abundance of the major histocompatibility complexes I and II (MHC-I, MHC-II), affinity of T cells for NAs, and the presence of driver mutation in the gene encoding NAs, have to be taken into account [41]. It is well known that the NA-vaccine efficacy results largely from the tumor mutational burden (TMB), i.e., the number of mutations per DNA fragment with the length of 1 million base pairs, but it can be limited due to low TMB values of some MNs. It should be remembered that TMB is considered to be a predictive biomarker for melanoma and NSCLC only [41, 42]. It was noted that the cultured DCs or DCs isolated from the patient's blood can be easily loaded with NAs using the routine procedures: electroporation or lentiviral transduction [43]. This contributes to the development of the mixed DC-NA-vaccines that have already shown their anticancer efficacy in the PCTs involving the models of PC, BRCA, NSCLC, CRC and Merkel cell carcinoma. Some of these vaccines are being studied in CTs (Table 2).

CONCLUSION

The DC- and NA-vaccines represent an intensively developed branch of the high-techbiotherapeutic anticancer drugs for the personalized application. Since certain technological aspects of the DC- and NA-vaccine preparation are characterized by considerable duration, high labor and resource intensity, optimization of preclinical developments aimed at accelerating,simplifying and cost reducing the DC-vaccine manufacture processis relevant. These developments will significantly increase the scale of the DC- and NA-vaccines applications in the future.

The approach directed totargeting the vaccines to cancer stem cells (CSCs) and their NAs seems to be ambitious and promising. According to a number of studies, tumors with aggressive phenotypes can contain large populations of CSCs that determine high proliferative potential and the disease progression [44]. However, a more detailed investigation of the CSC molecular genetic profile and the spectrum of the CSC specific biomarkers is needed to improve this approach.

Since the DC- and NA-vaccines have proved to be effective against a number of similarmalignant neoplasms, clinical assessment of the mixed (combined) NA-DC-vaccines should be considered as a promising area, however, the results of such CTs have not yet been published.

According to the analysis of the completed and active CTs, the combinations of DC-vaccines and ICIs currently demonstrate the highest anticancer efficacy along with acceptable safety and tolerability in patients with solid malignant neoplasms.

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