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Chapter

Immune and Cell Cycle Checkpoint Inhibitors for Cancer Immunotherapy

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Abstract

The rational design of immunotherapeutic agents has advanced with a fundamental understanding that both innate and adaptive immunity play important roles in cancer surveillance and tumor destruction; given that oncogenesis occurs and cancer progresses through the growth of tumor cells with low immunogenicity in an increasingly immunosuppressive tumor microenvironment. Checkpoint inhibitors in the form of monoclonal antibodies that block cancer's ability to deactivate and evade the immune system have been widely indicated for a variety of tumor types. Through targeting the biological mechanisms and pathways that cancer cells use to interact with and suppress the immune system, immunotherapeutic agents have achieved success in inhibiting tumor growth while eliciting lesser toxicities, compared to treatments with standard chemotherapy. Development of "precise" bio-active tumor-targeted gene vectors, biotechnologies, and reagents has also advanced. This chapter presents ongoing clinical research involving immune checkpoint inhibitors, while addressing the clinical potential for tumor-targeted gene blockade in combination with tumor-targeted cytokine delivery, in patients with advanced metastatic disease, providing strategic clinical approaches to precision cancer immunotherapy.

Keywords: PD-1 inhibitor, CTLA4 inhibitor, DeltaRex-G, DeltaVax, NK cells, checkpoint inhibitors, cell cycle control, GMCSF

1. Introduction

The human immune system is an intricate network of cell types and signaling pathways that act in a concerted effort to ensure that when an immune response is elicited, it is directly proportional to the severity of the attack. Although this network exists to protect the body from foreign invasion, an overactive immune response can lead to immunopathogenesis and autoimmunity, thus it is crucial that there are mechanisms set in place to ensure this system remains tightly regulated [1]. The immune system achieves this strict regulation by engaging a complex system of checkpoint control pathways. These checkpoints act as metaphorical gateways that require a specific key, in the form of a protein or a small molecule, in order to initiate tightly regulated signaling pathways that prevent over-reactivity of an immune response through the binding of specific cell surface receptors. This process is known as peripheral tolerance [2]. Certain checkpoint pathways,

including those involving transmembrane protein receptors cytotoxic t-lymphocyte antigen 4 (CTLA-4) and programmed death 1 (PD-1), play pivotal inhibitory roles in T-cell activation. Specifically, the CTLA-4 checkpoint is designed to inhibit T-cells from becoming autoreactive during the beginning stages of T-cell activation, while the PD-1 checkpoint is part of a family of costimulatory receptors that, when bound to its ligand, inhibits T-cell proliferation [2].

Tumor cells exploit the process of peripheral tolerance as a way to evade immunological surveillance by mimicking inhibitory receptors that are normally expressed on the surface of antigen presenting cells [3]. Expressing these inhibitory receptors allows cancer cells to effectively downregulate an immune response by deactivating the T-cells they come into contact with. The development of genetically engineered immune checkpoint inhibitors (ICIs) to treat malignancies therefore has the potential to revive pre-existing immune responses that would have otherwise been suppressed by the cancer [4]. Immunotherapies have been developed over the past decade using monoclonal antibodies as checkpoint inhibitors, binding the inhibitory receptor on T-cells and blocking tumor cells from binding to these sites.

The first immune checkpoint inhibition therapies to enter clinical trials for patients with advanced cancer were two fully human CTLA-4 blocking antibodies, ipilimumab and tremelimumab. Clinical activity of the CTLA-4 blockade was most significant in advanced melanoma patients, leading to a 15% response rate that, for some patients, persisted for over 10 years after discontinuing therapy [5]. In 2010, a large Phase III trial was published showing ipilimumab to have significantly improved overall survival rates in patients with metastatic melanoma, compared to treatment with standard gp100, a synthetic peptide cancer vaccine, alone [6]. Ipilimumab has since been FDA approved in combination for the treatment of advanced renal cell carcinoma, microsatellite instability high (MSI-H) or mismatch repair deficient (dMMR) metastatic colorectal cancer, hepatocellular carcinoma, non-small cell lung cancer (NSCLC), and malignant pleural mesothelioma.

The PD-1 checkpoint pathway was the next to be targeted with antibody therapy. Similar to ipilimumab, the first nivolumab trials were also shown to be most efficacious in melanoma patients, although it is now approved not only for the treatment of melanoma, but also of non-small cell lung cancer (NSCLC), small cell lung cancer, malignant pleural mesothelioma, renal cell carcinoma, Hodgkin lymphoma, squamous cell carcinoma of the head and neck, urothelial carcinoma, MSI-H or dMMR metastatic colorectal cancer, hepatocellular carcinoma, and esophageal squamous cell carcinoma. A study assessing the efficacy of anti-CTLA-4 and anti-PD-1 combined therapy in melanoma patients showed even more significant results, with 53% of patients achieving an objective response, and ≥ 80% tumor reduction was reported in all patients [7].

Thus far, the only two immune checkpoint inhibitors that have been successfully brought to market are those that involve the PD-1/PD-L1 checkpoint and CTLA-4 checkpoint. These targets are within the adaptive immune system, but scientists are looking at the potential anti-tumor effects of exploring checkpoint targets within the innate immune system. Another target currently being investigated involves immune checkpoint inhibition within natural killer (NK) cell-mediated immunity. Cancer cells frequently downregulate their MHC expression, rendering T-cell mediated immunotherapy insufficient for killing these tumor cells. NK cell-mediated treatment can, in theory, compensate for this. As a first line of defense within the immune surveillance system, NK cells are quicker to become activated and will indiscriminately induce apoptosis in any cell lacking MHC-receptors.

Similar to the immune system, a checkpoint control system is also used to control the distinct phases of the cell division cycle in order to regulate cellular proliferation. Unrestrained cell division is a fundamental characteristic of oncogenesis, therefore

cell cycle checkpoint control is vital in preventing the development of cancer. The mechanism of action in this case of checkpoint control is site-specific protein phosphorylation executed largely by cyclin-dependent proline-directed protein kinases. For example, Cyclin D1 and CDK4/6 are downstream of growth-initiating signaling pathways which lead to cellular proliferation. Palbociclib, an inhibitor of cyclin-dependent kinases CDK4/CDK6 is approved for the treatment of HR-positive, HER2-negative advanced or metastatic breast cancer in combination with an aromatase inhibitor as initial endocrine based therapy in postmenopausal women or fulvestrant in women with disease progression following endocrine therapy [8].

Another example of an executive cell cycle regulatory protein is the cyclin G1 protein, product of the CCNG1 proto-oncogene: (i) identified as the prime molecular driver of "Cell Competence" (to proliferate), (ii) needed for quiescent cells to enter the G1 phase, subject to oncogene-addiction as a molecular survival factor [9]. Tumor-targeted gene therapy involving CCNG1 blockade was tested in a number of clinical trials over a decade ago, and has recently been revived for clinical use, upon analysis of long-term cancer-free survival data, as the first clinically validated tumor-targeted gene therapy vector of this kind. This genetic medicine, known as DeltaRex-G (Former names: Mx-dnG1, dnG1, Rexin-G), is a "retroviral expression vector displaying a cryptic/designer collagen-binding motif on its gp70 surface envelope, designed specifically for targeting abnormal (anaplastic) Signature (SIG) proteins in the tumor microenvironment and encoding a dominant-negative mutant construct (dnG1) of human CCNG1 (Cyclin G1)oncogene/survival factor [10]. Once administered intravenously, the DeltaRex-G nanoparticles (~100 nm) accumulate in cancerous lesions, where the transgene is expressed, using the tumor cell's replication machinery to translate a mutant, cytocidal protein that is specifically designed to block the Cyclin G1 pathways of cell competence and survival function, leading to active cancer cell death via apoptosis.

Herein, we discuss the current landscape of immune and cell cycle checkpoint inhibition by presenting a selected number of ongoing and past clinical research for advanced malignancies at the Cancer Center of Southern California (CCSC)/Sarcoma Oncology Research Center (SORC) in Santa Monica, California, in context and collaboration.

2. Ongoing clinical research

Ongoing clinical research is either investigator-initiated or company sponsored. In the case of investigator-initiated research, CCSC/SORC serves as the sponsor, conceives and designs the clinical protocol, and manages the entire clinical trial with or without funding by a pharmaceutical company, the FDA or the NIH. Company-sponsored research is developed, monitored, and funded by a pharmaceutical company.

2.1 Investigator initiated research

2.1.1 SAINT: An Expanded Phase II Study Using Safe Amounts of Ipilimumab, Nivolumab, and Trabectedin as First-Line Treatment of Advanced Soft Tissue Sarcoma (NCT03138161). Erlinda M. Gordon, Principal Investigator

2.1.1.1 Background & rationale

Soft tissue sarcomas comprise a rare, heterogenous category of malignancies originating from connective tissue, blood vessels or lymphatic tissue [11].

This group accounts for only 1% of adult cancers in the United States, but it has a higher mortality rate than testicular cancer, thyroid cancer, and Hodgkin lymphoma combined [12]. The most commonly used modalities of treatment for sarcoma have been surgery, radiation and chemotherapy. Currently, chemotherapy treatment options have been shown to slow down disease progression but are ineffective in keeping most patients from eventually developing recurrent and metastatic disease [13]. Once unresectable or metastatic, the majority of soft tissue sarcomas remain incurable with chemotherapy. Immune checkpoint blockades do not act directly on the cancer cell, thus they can theoretically be applied to the treatment of any type of solid tumor, including the rarest and most aggressive malignancies. The precedent set by the approval of immune checkpoint inhibition for the treatment of numerous cancer types provides a strong rationale for studying their effects on soft tissue sarcoma. Studies with ipilimumab and nivolumab have since been done showing promising results when used in patients with advanced soft tissue sarcoma [14]. The third drug in this trial is a marine-derived alkaloid, trabectedin, an FDA approved chemotherapy treatment for leiomyosarcoma and liposarcoma [15]. A recently published retroactive analysis of 442 patients treated with trabectedin over a 10 year period confirms that trabectedin can prolong progression free survival (PFS) in patients with advanced sarcoma [16].

Gordon et al. designed the SAINT protocol based on the fact that sarcoma cells are most immunogenic early in the disease process [17] and prior to any other treatment, allowing immune checkpoint inhibitors to exploit this advantage and deploy the immune system to recognize and destroy them. This study was designed to evaluate the best objective response rates (BORR) assessed via CT scan or MRI and to assess the overall survival (OS) and progression-free survival (PFS) after 6 months of treatment.

2.1.1.2 Methods

Eligible patients for this Phase II clinical trial were treatment-naïve adult patients with advanced unresectable or metastatic soft tissue sarcoma. Trabectedin was administered to the study subjects at the maximum tolerated dose determined previously in the dose escalation phase of this trial. Ipilimumab and nivolumab were be administered at defined doses in order to assess the overall safety profile and potential efficacy of this treatment regimen. Patients continued on the treatment until they experienced significant disease progression or unmanageable toxicities. Best objective response was measured according to Response Evaluation Criteria in Solid Tumors (RECIST) v1.1 using CT scan or MRI. Median progression-free survival (PFS) and overall survival (OS) were also measured in months. Adverse events were assessed and categorized as related or unrelated to the treatment and listed by severity according to the Common Terminology Criteria for Adverse Events (CTCAE) version 5.0.

2.1.1.3 Preliminary results were presented at the Connective Tissue Oncology Society Meetings, November, 2020

Sixty subjects were evaluated using RECIST v1.1 for analysis of treatment efficacy. Twenty-five percent (25%) had either a complete response (11.7%) or a partial response (13.3%), and 37 patients (61.7%) had stable disease. Disease control rate was 86.6%. The median PFS was >6.7 months (6-month OS rate: 90%; 6-month PFS rate: 51%), while the median OS was >17.0 months.

Grade 3 TRAEs included fatigue (n = 6), adrenal insufficiency (n = 1), hyperglycemia (n = 1), dehydration (n = 1), hyponatremia (n = 2), bipedal edema (n = 2),

increased AST (n = 6), increased ALT (n = 19), increased ALP (n = 1), port site infection (n = 2), psoriasis exacerbation (n = 1), anemia (n = 3), thrombocytopenia (n = 2), leukopenia (n = 1), and neutropenia (n = 3). Grade 4 TRAES include anemia (n = 1), neutropenia (n = 1), thrombocytopenia (n = 1), and increased CPK (n = 2). Grade 5 TRAES include rhabdomyolysis (n = 1). Therapy related AML occurred in one patient.

2.1.1.4 Conclusions/future directions

The positive results from this trial thus far strongly suggest that using combination therapy with ipilimumab, nivolumab, and trabectedin as first-line treatment in patients with advanced or metastatic sarcoma allows the treatments to engage synergistically without causing any additive toxicities. This combination may be superior to known therapies for STS. Overall, the adverse events experienced less severe than toxicities typically experienced with standard first line treatment (doxorubicin/ifosfamide) for metastatic soft tissue sarcoma. Future Phase 3 randomized studies are proposed to evaluate the safety and efficacy of first-line combinatorial therapy with ipilimumab, nivolumab and trabectedin in comparison to standard therapy for patients with advanced soft tissue sarcomas.

2.1.2 The TNT Protocol: A Phase II Study Using Talimogene Laherparepvec, Nivolumab, and Trabectedin as First, Second/Third Line Therapy for Advanced Sarcoma, including Desmoid Tumor and Chordoma (NCT03886311). Sant P. Chawla and Erlinda M. Gordon, co-Principal Investigators

2.1.2.1 Background & rationale

The significant immunotherapeutic potential of oncolytic virotherapy is due to its ability to induce a multifaceted anti-tumor response involving aspects of both the innate and adaptive immune systems [18]. A multitude of viral vectors have been explored for their potential oncolytic properties, particularly as a method of delivering targeted treatment to sites of malignant disease [19]. The ability to genetically modify these viruses to target and exploit essential oncogenic signaling pathways has kept them at the forefront of immuno-oncology research [20]. This particular vulnerability triggers selective replication of the viral genome and directly contributes to furthering the oncolytic process. Infected tumor cells secrete viral progeny composed in part by tumor-associated antigens and neoantigens in response to their infection, causing the innate immune system to activate an NK cell-mediated cytotoxic response. The tumor-associated antigens that are released into the tumor microenvironment are phagocytosed by antigen-presenting cells, thus initiating the process of T-cell-mediated adaptive anti-tumor immunity. In addition to the anti-tumor response, the presence of the oncolytic virus also triggers a concurrent anti-viral response, and regulatory mechanisms become crucial to ensuring a controlled immune response, including the upregulation of immune checkpoints [20].

Oncolytic viruses derived from Herpes simplex virus 1 (HSV-1) vectors are amongst the most frequently investigated in pre-clinical trials and have been shown to encompass the combined ability to induce oncolysis and anti-tumor immune responses simultaneously [21]. Talimogene Laherparepvec (T-VEC) is an injectable live, attenuated, oncolytic HSV-1 virus that has been genetically engineered to express human granulocyte-macrophage colony-stimulating factor (huGM-CSF), a known immune modulator and hematopoietic growth factor that stimulates the

differentiation of multipotent progenitor cells and plays a key role in the functional abilities of many different circulating lymphocytes, including T-cells [22].

The objective of this ongoing study is to evaluate the potentially synergistic effects T-VEC may evoke when used in combination with anti-PD-L1 monoclonal antibody, nivolumab and marine derived alkaloid, trabectedin. The study is ongoing.

2.1.2.2 Methods

This open-label Phase II study is designed to assess the safety and efficacy of this combination treatment. This will be accomplished by determining the median month of progression-free survival, median duration of response, and best overall response rates based on each patients' percent of change in their tumor sizes. This study plans to enroll 40 participants with advanced disease who have at least one tumor that is easily accessible for intratumoral injection with T-VEC. Regarding the statistical analysis, continuous variables will be summarized by the sample size (n) and measures of central tendency and variation will be calculated including mean, standard deviation, first and third quartiles, maximum and minimum. Categorical variables will be summarized by the sample and by the percent in each category. "Point estimates for efficacy endpoint incidences will be accompanied by a 2-sided 95% exact binomial CI. Time to event endpoints will be summarized descriptively using the KM method. Safety (incidence and severity of adverse events and significant laboratory abnormalities) will be performed on all patients (ITT population). Patient incidence of all treatment emergent AEs will be tabulated by system organ class and preferred term" [23].

2.1.2.3 Preliminary results presented at the Connective Tissue Oncology Society meetings, November, 2020

Efficacy analysis (n = 31): There were 6.5% partial responses, 80.6% disease control rate, with 74.1% PFS rate and 92.6% OS rate at 4 months.

Safety Analysis (n = 41): Grade 3 TRAEs include fatigue (n = 2), decreased ejection fraction (n = 1), anasarca (n = 1), dehydration (n = 1), decreased cortisol (n = 1), anemia (n = 9), thrombocytopenia (n = 4), neutropenia (n = 4), gastroenteritis (n = 1), increased ALT (n = 8), increased AST (n = 1), and increased GGT (n = 1). Grade 4 TRAEs observed were thrombocytopenia (n = 2). There was no conversion from unresectable to resectable tumor. There were thirty-one evaluable subjects for efficacy analysis.

2.1.2.4 Conclusions/future directions

Second- or third- line combinatorial therapy with talimogene laherparepvec, nivolumab, and trabectedin.

1. may be equal or better than standard second/third line therapy in achieving disease control.

2. may be safer than standard therapy for patients with advanced soft tissue sarcoma with no unexpected toxicities.

2.1.2.4.1 Future directions for research

Studies are proposed to evaluate the efficacy and safety of first, second/third line combinatorial therapy with talimogene laherparepvec, nivolumab and

trabectedin vs. standard therapy (doxorubicin/ifosfamide) in randomized studies for advanced soft tissue sarcomas.

2.1.3 A Phase Ib investigation of safety/efficacy of nivolumab and ABI-009 (nab-rapamycin) in advanced undifferentiated pleomorphic sarcoma (UPS), liposarcoma (LPS), chondrosarcoma (CS), osteosarcoma (OS) and Ewing sarcoma (NCT03190174). Erlinda M. Gordon, Principal Investigator

2.1.3.1 Background & rationale

Aberrant mTOR signaling, typically due to either an activating mutation in oncogenes related to the mTOR pathway or a loss of function mutation in an upstream tumor suppressor gene, has been found to play a significant, multifaceted role in oncogenesis [24, 25]. Originally discovered while investigating the targets of the drug rapamycin, a potent immunosuppressive agent, in the early 1990's, the protein kinase, mammalian target of rapamycin (mTOR), is a major signaling hub for directing cellular growth, metabolism, and proliferation [26]. While studying the mechanism of action behind rapamycin's inhibitory effects on mTOR signaling, the drug was also found to be involved in the inhibition of T-cell proliferation, specifically between the G_1 and S phases of the cell cycle. This finding launched a multitude of studies to better understand the role of mTOR signaling in T-cell activation and proliferation [27], culminating in the discovery that T-cells are also highly dependent on mTOR signaling to maintain normal T-cell activation and proliferation [28]. When t-cells receive immune stimuli, they then rely on signals from mTOR to promote t-helper cell differentiation while simultaneously inhibiting the induction of regulatory T-cells. Thus, mTOR exerts control over essential regulatory signals in both adaptive and innate immunity.

Initial clinical studies investigating the anti-cancer effects of single agent mTOR inhibitor, were disappointing, reporting its limited effects, thus leading to the investigation of rapamycin in combination with various chemotherapy agents where it was successful in inhibiting cancer growth in prostate cancer patients [29]. However, the experimental drug, ABI-009 or nab-sirolimus, a novel albumin-bound mTOR inhibitor, has been shown to be effective and safe for the treatment of malignant perivascular epithelioid cell tumors (PEComa) [30]. A phase 1/2 trial is currently ongoing to investigate the potential synergistic activity of nab-sirolimus when administered with an immune checkpoint inhibitor, nivolumab, in improving clinical outcomes for patients with advanced sarcoma.

2.1.3.2 Methods

The original objectives of the study are: (1) To investigate the maximum tolerated dose (MTD) of ABI-009 when given with nivolumab, a PD-1 inhibitor, in previously treated advanced undifferentiated pleomorphic sarcoma, liposarcoma, chondrosarcoma, osteosarcoma and Ewing sarcoma; (2) To investigate the disease control rate (DCR), progression-free survival (PFS), and overall survival (OS) of this combination therapy in the aforementioned patient group, and (3) To correlate PFS with PD-L1 and other biomarker expression in patients' tumors.

This is an IRB approved protocol with 2 parts. The phase 1 part is a dose-finding study using the "cohort of three design", wherein a standard dose of nivolumab 240 mg is given IV every 3 weeks (day 1 of every 21-day cycle). Escalating doses of ABI-009 are given IV on days 8 and 15 of each cycle starting in Cycle 2 following the 2nd nivolumab dose. The starting dose of ABI-009 is 56 mg/m^2, and sequentially escalating doses are 75, and 100 mg/m^2. Phase 2 of the study will enroll 31 additional patients to further assess efficacy and safety at the MTD.

2.1.3.3 Preliminary results presented at the ASCO annual meeting in June, 2019

The Phase I part of study included 9 patients who were treated successfully at 3 dose levels. No dose-limiting toxicities (DLTs) were observed, the MTD was not reached, and 100 mg/m^2 ABI-009 was designated as the recommended Phase II dose. Safety analysis: At Dose 1 (n = 3): Grade 3 treatment-related adverse events (TRAEs) included dyslipidemia (n = 1) and hyperglycemia (n = 1). At Dose 2 (n = 3): Grade 3 TRAEs included increased ALT (n = 1). At Dose 3 (n = 3): Grade 3 TRAEs included hypophosphatemia (n = 1).

2.1.3.4 Conclusions/future directions

The primary endpoint has been met with no DLTs, the MTD was not reached and Dose 3 (100 mg/m2) of ABI-009 has been designated as the phase 2 dose which is on-going.

2.2 Company sponsored clinical research

2.2.1 Phase I Study of INBRX-109 in Subjects With Locally Advanced or Metastatic Solid Tumors Including Sarcomas (NCT03715933) Sant P. Chawla, Principal Investigator

2.2.1.1 Background & rationale

The initiation of the extrinsic apoptosis pathway is mediated by several death domain receptors including death receptor 5 (DR5), a transmembrane protein receptor activated by the tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) [31]. The DR5 apoptosis pathway naturally occurs to rid the body of neoplastic cells and is known to be crucial in immune system surveillance against cancer growth and metastasis. Normally, when an anchorage-dependent cell becomes detached, the cell will undergo a process of detachment-induced apoptosis called anoikis, initiated by a death receptor-mediated apoptotic pathway. A reduction in DR5 expression was found in melanoma tumor samples, strongly implying the TRAIL/DR5 pathway is associated with the prevention of tumor metastasis [32]. In 1999, Walczak et al. observed tumor cells to have a significantly higher sensitivity to TRAIL than normal cells, emphasizing its potential as a therapeutic cancer agent [33]. The subsequent development of agonistic antibodies against DR5 (i.e. recombinant human TRAIL proteins) was shown to be successful in stimulating apoptosis when tested in various tumor cell lines, and was later also shown to enhance the efficacy of chemotherapy and radiotherapy [34].

INBRX-109 is a tetravalent DR5 agonist antibody designed to initiate the DR5 apoptosis pathway and precisely engineered to avoid unnecessary crosslinking to lower the risk of hepatotoxicity.

2.2.1.2 Methods

This is the first in-human, open-label, non-randomized Phase I clinical trial for INBRX-109. Eligible patients had metastatic or unresectable solid tumors refractory to standard treatment or for which there is no FDA approved standard treatment. Phase I consists of two parts, part 1 being a 3 + 3 dose escalation cohort and part 2 being a dose expansion cohort. Safety, tolerability and dose-limiting toxicity were measured and analyzed using the National Cancer Institute's Common Terminology

Criteria for Adverse Events (CTCAE) criteria to assess the severity of adverse events experienced. This study's exploratory objective is an assessment of anti-tumor activity and was reported according to RECIST v1.1 standard.

2.2.1.3 Preliminary results presented at the CTOS meeting, November, 2020

Overall, INBRX-109 was well tolerated and approximately 90% of patients showed no signs of hepatotoxicity. The pharmacokinetics of INBRX-109 were approximately dose proportional across all doses tested and thus support dosing every 3 weeks without administration of any premedications. All patients have thus far tested negative for anti-drug antibodies. Maximum tolerated dose was not reached in the dose escalation cohort and only one dose-limiting toxicity was experienced. Very few serious adverse events that occurred were attributable to the drug being studied. One patient with mesothelioma has been reported to have experienced acute hepatic failure leading to death that could possibly be related to the study drug. Evidence of anti-cancer effects were observed in patients with chondrosarcoma, resulting in durable partial responses and stable disease.

2.2.1.4 Conclusions/future directions

NBRX-109, a precisely engineered tetravalent DR5 agonist antibody, showed promising results that warrant further exploration. The pharmacokinetics of INBRX-109 were essentially dose-proportional across all three dose levels, supporting dosing every three weeks with no premedications necessary. Specifically, the chondrosarcoma cohort of this Phase I study has been expanded to include twenty patients and is currently ongoing.

2.2.2 An Open-labeled, Phase I Study to Evaluate the Safety and Tolerability of Apatinib with Nivolumab in Patients with Unresectable or Metastatic Cancer (NCT03396211) Sant P. Chawla, Principal Investigator

2.2.2.1 Background & rationale

Unregulated angiogenesis is one of the key characteristics of malignant tumors [35]. In addition to creating neovasculature, tumor angiogenesis plays a key role in creating an immunosuppressive tumor microenvironment by causing an accumulation of pro-tumor immune cells and a decrease in the abundance and function of anti-tumor immune cells. Anti-angiogenic cancer treatments have been shown to reverse this process, essentially 'reprogramming' the tumor microenvironment by converting it from an immunosuppressive to an immunogenic one. This has been accomplished by targeting and inhibiting vascular endothelial growth factor (VEGF), a well-known cell surface-signaling proangiogenic protein that becomes stimulated when bound to tyrosine kinase receptors. With the use of antiangiogenic small molecule tyrosine kinase inhibitors (TKIs), VEGF can be blocked from binding its receptor, stopping the tumor from being able to continue to create neovasculature [36]. However, cancer has been able to circumvent this blockade using multiple other pathways, suggesting the use of antiangiogenic therapies that inhibit more than one signaling pathway simultaneously.

The experimental drug, apatinib, is a highly selective VEGFR-2 TKI, administered orally, that has already been approved in China for ≥3rd-line treatment for advanced gastric cancer. The potential benefit of combining TKI and PD-1 therapies has been demonstrated in preclinical murine models, suggesting that

combining ICIs with antiangiogenesis therapy could have the synergistic antitumor effects needed to enhance the efficacy of the individual therapies [37]. This phase 1 single-center clinical trial beginning in 2018 evaluated the safety and tolerability of TKI, apatinib, and PD-1 inhibitor, nivolumab, in patients with unresectable or metastatic cancer.

2.2.2.2 Methods

All subjects enrolled had cancer that was refractory to prior lines of treatment. Specifically, patients were required to already be at least three cycles into nivolumab treatment and must be planning to continue this treatment throughout the trial period. Thirty patients in total were enrolled, ten of which were in part 1 of this study, where they were treated with apatinib using a classic 3 + 3 dose escalation method in order to determine the maximum tolerated dose. Part 2 of this study included twenty subjects and was an expansion phase using the MTD. The percent of change in tumor responses and amount of time until progression were measured and analyzed using RECIST v1.1 and iRECIST criteria.

2.2.2.3 Preliminary results presented at the Connective Tissue Oncology Society meetings, November, 2020

The overall response rate reported was 13.3% (95% CI: 3.8% to 30.7%) and the median PFS was 7.2 months (95% CI: 5.3 to 9.0 months). No complete responses occurred although four patients achieved a partial response and the majority of patients achieved stable disease. Seven patients from part 1 and six patients from part 2 experienced ≥ grade 3 treatment emergent adverse events including fatigue (10.0%), hypertension (10.0%), nausea (10.0%), anemia (16.7%) and asthenia (10.0%). Two patients experienced fatal adverse events, although there were no noted treatment related deaths. Nine patients eventually discontinued the study due to toxicity, and nine patients also received a dose reduction. No unexpected side effects were noted as a result of the combined treatment.

2.2.2.4 Conclusions/future directions

The results of this study demonstrate an acceptable safety profile and clinical benefit of combination treatment with nivolumab and apatinib that is worth exploring further in additional clinical studies.

2.2.3 Phase 1, Open-Label, Safety Study of Escalating Doses of Ex Vivo Expanded, Autologous Natural Killer Cells in Patients With Pathologically Confirmed Cancer Refractory to Conventional Therapy (NCT03941262). Sant P. Chawla, Principal Investigator

2.2.3.1 Background & rationale

Natural killer (NK) cells are the cytotoxic lymphocytes of the innate immune system [38]. As a rapid, first line of defense, NK cells are able to lyse tumor cells independent of the expression of tumor-associated antigens and/or the presence of major histocompatibility complex class I (MHC-I) molecules. This ability is crucial as cancer cells have been shown to downregulate the expression of MHC-I on their cell surface as a way to evade detection by immunosurveillance mechanisms [39].

Since the expression of MHC-I on cancer cells is needed for their detection and destruction by T-cells, evolving a loss of MHC-I expression has been a way for tumor cells to remain undetectable and this loss has therefore been reported "as a mechanism of resistance to anti–PD-1 therapy" [40]. In order to avoid the development of resistance to PD-1 checkpoint inhibition therapy, exploration of NK cell therapy is warranted, especially because of the NK cell's specific role in the recognition and destruction of cancer cells that display a loss of MHC-I. Additionally, the broad ability of NK cells to destroy tumor cells irrespective of prior sensitization or immunization therapy make them ideal candidates for engineered cell therapies.

Discoveries regarding the NK cell's role in anti-tumor immunity coupled with advancements in the field of hematopoietic stem cell transplantation have brought to light the potential in using NK cell-mediated immunotherapeutic strategies to fight cancer [41]. Adoptive immunotherapy using donor-derived autologous NK cell products can be engineered by using monoclonal antibodies alone, or in combination with in vivo and ex vivo NK cell activation techniques [42]. This is done by obtaining a patient's NK cells and incubating them as highly active NK cells, giving them the ability to mass produce NK cells which are then infused back into the patient. This study explores the safety and tolerability of treating cancer with SNK01 (autologous natural killer cells).

2.2.3.2 Methods

This study is a nonrandomized, multicenter safety study of adoptively infused, ex vivo expanded autologous NK cells to treat male and female adult patients with advanced or metastatic, intractable cancer. Study subjects were placed in one of three cohorts and received SNK01 in an open-label fashion according to a 3 + 3 Phase 1 dose-escalation method. Patients received 5 weekly infusions for a period of 5 weeks, and restaging imaging was performed on week 6. The primary objective of this study was a safety assessment based on the incidence and severity of dose-limiting toxicities and other adverse events observed by evaluating vital signs, clinical laboratory findings and physical examination abnormalities. The adverse events were graded according to the CTCAE v5.0 criteria. Subjects' performance status was assessed and recorded using ECOG criteria. The secondary objective is to evaluate the efficacy of the treatment by measuring the objective response rate of target lesions observed via CT scan using iRECIST criteria.

2.2.3.3 Preliminary results

Not yet available.

2.2.3.4 Conclusions/future directions

In order to effectively achieve immune surveillance, immunosuppressive signals within the tumor microenvironment must be interrupted. The PD-1/PD-L1 signaling blockade was developed in accordance with this principle. Tumors have been shown to secrete cytokines associated with suppression of T-cells and NK cells, and past murine studies have shown circulating IL-18 in low levels originating from tumor cells can suppress NK anti-tumor activity [41]. The principles of checkpoint blockade can be applied here with the development of a neutralizing antibody to IL-18, suggesting the potential of checkpoint inhibition to improve in vivo NK cell activity.

3. Past clinical research

3.1 Immune Design: A Randomized, Open-Label, Phase II Trial of CMB305 (Sequentially Administered LV305 [lentiviral vector expressing NYESO-1 gene] and G305[NY-ESO-1 recombinant protein plus GLA-SE]) and Atezolizumab in Patients with Locally Advanced, Relapsed, or Metastatic Sarcoma Expressing NY-ESO-1 (NCT02609984) Sant P. Chawla, Principal Investigator

3.1.1 Background & rationale

NY-ESO-1 is a protein that is normally expressed in fetal and testicular tissues, although some solid malignancies have been known to express an abnormal NY-ESO-1 protein that has become a target for emerging antigen-directed cancer therapies [43, 44]. Previous studies looking at NY-ESO-1 expression in cancer cells have reported its presence in the majority of synovial sarcomas tested, as well as sporadic expression in a number of other sarcoma subsets [45]. The immunogenicity of NY-ESO-1 has been demonstrated by the discovery of receptors against NY-ESO-1 on $CD8^+$ T-cells. A 2011 clinical trial conducted by the National Cancer Institute was the first to report promising anticancer effects of NY-ESO-1-targeted immunotherapy in patients with metastatic synovial sarcoma using adoptively transferred autologous T-cells containing a T-cell receptor against NY-ESO-1 [46], suggesting its potential to be effective in other sarcomas as well. Since then, numerous trials targeting NY-ESO-1 in various cancer types using both adoptive T-cell therapy and vaccination approaches have concluded that there is a clear clinical benefit in pursuing NY-ESO-1 as an immunotherapeutic target [47].

The drug being studied is CMB305, a prime-boost immunotherapeutic vaccine regimen developed to prime the immune system and enhance its subsequent response to immunotherapeutic agents. The priming component of CMB305 is an integration-deficient, replication-incompetent lentiviral vector containing RNA coding for NY-ESO-1. The boost component contains a recombinant E. coli-produced NY-ESO-1 protein that, as a single agent, can initiate anti-NY-ESO-1-specific $CD4^+$ T-cell and antibody responses. The combination of the primer and the booster was designed with the intention of eliciting an enhanced T-cell response.

The goal of this study was to investigate the ability of a prime-boost immunotherapy regimen that is able to elicit NY-ESO-1-specific $CD8^+$ T-cells to synergistically enhance the efficacy of PD-L1 checkpoint inhibition therapy in advanced or metastatic sarcoma patients whose tumors are positive for NY-ESO-1 expression.

3.1.2 Methods

The primary objective of this study was to compare the progression-free survival in locally advanced or metastatic sarcoma patients whose tumors expressed NY-ESO-1 when treated with CMB305 in combination with atezolizumab versus patients treated with atezolizumab alone. The secondary objectives of this study were to evaluate the safety of this combination treatment, as well as to evaluate the best overall response rate using RECIST v1.1 modified to use immune-related response criteria. The overall survival of the two groups will be evaluated.

Twelve patients were randomized 1:1 in a safety run-in evaluation. Next, 80 patients were randomized and stratified by disease. Tumor samples from all patients were tested for levels of PD-L1 and NY-ESO-1 expression prior to treatment, and again on Day 42 in order to assess the extent of successful immune cell invasion in the tumor. Re-staging imaging studies were performed every six

weeks for the first twelve months, followed by staging every twelve weeks until the patient displayed symptomatic progression. CMB305 treatment was administered in seven doses over a three-month period, while atezolizumab was administered intravenously every three weeks, and was continued up to two years or until toxicities began to develop. An additional booster dose was also given every six weeks for the first year or until the patient displayed disease progression. Blood samples were collected to test for lentivirus vector persistence at baseline, six, twelve and twenty-four months following the initial treatment. Adverse events were recorded as related or unrelated to the study drug and graded based on CTCAE c4.03 criteria.

3.1.3 Published results

Not Available.

3.1.4 Conclusions/future directions

Phase I of this trial was the first of its kind to test a prime-boost vaccination regimen to treat patients with advanced cancer. In 2018, Immune Design released information stating that an early analysis of the Phase II clinical trial results showed the combination treatment of atezolizumab with CMB305 suggested that it is unlikely this regimen will show enhanced survival time of patients with recurrent synovial sarcoma [48]. A Phase III trial has not yet been pursued.

3.2 A Phase I-II Study Using DeltaRex-G (Former name:Rexin-G)Tumor-Targeted Retrovector Encoding a Dominant-Negative Cyclin G1 Inhibitor for Advanced Pancreatic Cancer (NCT00504998) Sant P. Chawla, Michael Morse, Howard Bruckner, Principal Investigators

3.2.1 Background & rationale

Advanced pancreatic adenocarcinoma is the third most common cancer type in the Unites States, although diagnostic tests are non-specific which leads to early-stage disease frequently going undetected [49, 50]. Once pancreatic adenocarcinoma reaches an advanced stage, it has likely become intractable and there is no cure. Previous targeted therapies revolved around the epidermal growth factor receptor (EGFR) signaling pathway, one of the most significant factors regulating cell growth, survival, differentiation and proliferation, making it a promising target for precision medicine [51]. EGFR signaling has been identified as an oncogenic driver in multiple cancer types, and EGFR inhibitors have been used as targeted therapy for pancreatic cancer [52].

DeltaRex-G is the first injectable tumor-targeted gene delivery system to be developed for cancer that blocks the G1 checkpoint of the cell division cycle of cancer cells by inhibiting the CCNG1 gene. DeltaRex-G includes a mutant construct of the CCNG1 gene that inhibits human cyclin G1, a proto-oncogene that promotes cell competence, cell survival, and stem cell proliferation. When administered systemically, DeltaRex-G seeks out and accumulates in tumor tissues by binding abnormal collagenous signature (SIG) proteins that are characteristically exposed as anaplasia during tumor invasion. Once the DeltaRex-G retrovector is incorporated in rapidly dividing cells, a cytocidal CCNG1 inhibitor protein is expressed that effectively blocks the cell division cycle, resulting in apoptosis and subsequent eradication of cancer cells, proliferative vasculature, and stroma.

Clinical data from DeltaRex-G trials conducted initially in the Philippines showed promising results for patients with advanced pancreatic adenocarcinoma.

This prompted USFDA Orphan Drug status, leading to progressive clinical trials in the United States, using DeltaRex-G to treat chemotherapy-resistant advanced pancreatic adenocarcinoma, soft tissue sarcoma, osteosarcoma, and breast cancers. This study reports the results compiled from a Phase I-II clinical trial using intravenous infusions of DeltaRex-G as treatment for advanced pancreatic cancer.

3.2.2 Methods

Twenty patients with chemotherapy-resistant metastatic pancreatic cancer were enrolled in the trial. Target lesions were identified in each patient and changes in tumor size were measured using RECIST v1.0 criteria. Patients were grouped and treated at 3 escalating doses of DeltaRex-G, with six patients at Dose 0-I, seven patients at dose level II, and seven patients at dose level III. Fifteen patients completed at least one full 4-week treatment cycle and had a follow-up PET-CT scan. These fifteen subjects comprised the modified intent-to-treat (mITT) population and were evaluated in terms of their response to the treatment, months of progression-free survival and months of overall survival.

3.2.3 Published results

The safety analysis revealed no clinically significant dose-limiting toxicities at any of the 3 dose levels, with no serious adverse events related to the study drug. None of the patients tested positive for vector neutralizing antibodies, replication-competent retrovirus in peripheral blood lymphocytes, antibodies to gp70, or vector integration into the genomic DNA of peripheral blood lymphocytes. According to the RECIST v1.0 evaluations of tumor responses, one patient achieved a complete response, two patients, partial response, and 12 patients, stable disease with 100% disease control rate. The median progression free survival by RECIST v1.0 was 2.7 months, 4.0 months, and 5.6 months at Dose levels I, II, and III, respectively. Median overall survival was 4.3 months, 9.2 months, and 9.2 months at Dose levels I, II, and III, respectively. A dose response relationship was shown between duration of survival and DeltaRex-G dosage ($p = 0.03$). Consequently, fast track designation was given by the USFDA for a planned Phase 2/3 study using DeltaRex-G as second line therapy for advanced pancreatic adenocarcinoma.

3.2.4 Conclusions/future directions

DeltaRex-G is a potent cytotoxic cell cycle checkpoint inhibitor. Complete and partial responses were observed at dose levels II and III, suggesting a significant dose–response relationship between the dose of DeltaRex-G given and the level of response seen in the tumors. This relationship is further implied by the increase in months of progression free survival as the dosages were increased. Additionally, CCNG1 is expressed in over 50% of various different malignancies other than pancreatic cancer, suggesting DeltaRex-G's potential efficacy in other cancer types [10].

3.3 Immune cell trafficking in the tumor microenvironment of human cyclin G1 (CCNG1) inhibitor-treated tumors

3.3.1 Background & rationale

Cell cycle checkpoint pathways that govern uninhibited cell proliferation can be rendered ineffective by a variety of cancer-induced immunosuppressive mechanisms [53]. The experimental cancer gene therapy, DeltaRex-G, is a pathotropic

(disease-seeking) retrovector designed to disrupt the cell cycle machinery of proliferative tumor cells, forcing them to undergo apoptosis. This is accomplished through "precise" tumor-targeted gene delivery to block the Cyclin G1/Cdk/cMyc/Mdm2/p53 Axis, effectively arresting the dividing tumor cell in G1 phase of the cell cycle undermining CCNG1 oncogene addiction. Clinical trials using DeltaRex-G to treat cancers that are unresponsive to traditional therapy have shown remarkable efficacy in evoking long term cancer-free survival with monotherapy (>10 years) in a number of patients with pancreatic cancer, osteosarcoma, soft tissue sarcomas, breast cancer, and B-cell lymphoma [54]. Although DeltaRex-G is involved in cell cycle checkpoint inhibition, it has also been shown to reduce extracellular matrix production by tumor cells and increase immune cell entry into the tumor microenvironment, which raises the clinical potential for DeltaRex-G to work synergistically with specific immune checkpoint inhibitors.

One persistent thought is that blanket recruitment of immune cells to the tumor microenvironment may not always be advantageous in creating an effective anti-tumor response. Certain tumor-infiltrating immune cells of myeloid origin have been shown to aid in tumor metastasis [55]. Cancers often progress and metastasize using immunosuppressive mechanisms that includes production and secretion of molecules that recruit cells involved in immune responses to the tumor microenvironment, and by exploiting checkpoint altering pathways [56]. Alternatively, and plausibly, this is how the innate immune system works in a healthy individual, with its molecular start and stop switches to prevent exaggerated immune responses and autoimmune disease. This study reviews published literature on the specific tumor-infiltrating immune cells seen in tumors of patients treated with DeltaRex-G.

3.3.2 Methods

A review of published literature was conducted on articles pertaining to the efficacy of DeltaRex-G in influencing the tumor microenvironment. The tumor types identified throughout the literature review included pancreatic adenocarcinoma metastatic to the liver, melanoma metastatic to the inguinal lymph node, colorectal cancer metastatic to the lungs, pancreatic B-cell lymphoma metastatic to the liver and cervical lymph nodes, recurrent breast ductal adenocarcinoma, and non-small cell lung carcinoma metastatic to the adrenal gland. The presence of tumor-infiltrating lymphocytes in excised tumors of patients treated with DeltaRex-G was assessed using immunohistochemical staining, and anti-tumor immune cells were differentiated from pro-tumor immune cells by their cytological characteristics. Agents included in the category of anti-tumor immune cells included dendritic cells, helper T-cells, natural killer cells, and killer T-cells. Regulatory T-cells and B-cells have the ability to encourage tumor growth by preventing antigen presentation and killer T-cell activation, thus were categorized as possibly pro-tumor immune cells. M1 macrophages were categorized as anti-tumor, although M2-type tumor-associated macrophages can promote tumor pathogenicity by overpowering M1-type tumor-infiltrating macrophages that elicit anti-tumor inflammation and were therefore categorized as pro-tumor. Results were reported based on cancer type.

3.3.3 Published results

Killer T-cells were identified in the tumor microenvironment of all cancers analyzed and helper T-cells were identified in all tumor types except for pancreatic B-cell lymphoma metastatic to the liver and cervical lymph nodes. Dendritic cells were found in metastatic pancreatic adenocarcinoma, metastatic melanoma, breast

ductal adenocarcinoma and metastatic non-small cell lung cancer. Natural killer cells were seen in metastatic pancreatic adenocarcinoma and metastatic non-small cell lung cancer. M1 macrophages were seen in breast ductal adenocarcinoma.

B-cells, possible pro-tumor cells, were seen in metastatic pancreatic adenocarcinoma, metastatic colorectal cancer, breast ductal adenocarcinoma and metastatic non-small cell lung cancer. Leukocyte common antigen was seen in metastatic pancreatic adenocarcinoma, metastatic melanoma, and non-small cell lung cancer. Pro-tumor macrophages were seen in breast ductal carcinoma.

3.3.4 Conclusions/future directions

Activating and optimizing the body's own immune system is at the core of precision medicine. Pathologic review showed evidence of **enhanced immune cell trafficking in the tumor microenvironment of patients treated with DeltaRex-G, suggesting that this treatment activates the innate immune response**. This implies that DeltaRex-G may enhance the performance of an immunotherapy agent when used simultaneously. Three patients identified in the literature with metastatic pancreatic cancer, B-cell lymphoma and metastatic osteosarcoma, respectively, have survived over 10 years following treatment with DeltaRex-G, no cancers have recurred, and no additional treatments have been needed. This clinical evidence strongly suggests that **DeltaRex-G has the ability to promote cancer immunization in situ through CCNG1 inhibition without causing deleterious immune suppression** [9]. Therefore, further evaluation of the role of DeltaRex-G in enhancing immune cell trafficking in the tumor microenvironment is warranted.

3.4 The Genevieve Protocol: Phase I/II Evaluation of a Dual Targeted Approach to Cancer Gene Therapy/Immunotherapy. Jorge G. Ignacio, Principal Investigator

3.4.1 Background & rationale

Patients whose cancer has recurred or progressed after therapy have likely exhausted their treatment options [57]. This is where the need for research towards the development of personalized targeted treatments becomes both vital and urgent. The GeneVieve (Genes for Life) Protocol was a dose-seeking study for chemoresistant solid malignancies and B-cell lymphoma, that evaluated the efficacy and safety profile of a dual targeted gene therapy regimen using DeltaRex-G and DeltaVax (Former name: Reximmune-C), two personalized vaccination strategies aimed to augment immune cell trafficking within the tumor microenvironment for in situ autoimmunization. DeltaRex-G is a retrovector encoding a cytocidal "dominant-negative" mutant construct of the human CCNG1 (Cyclin G1) oncogene. This retrovector is designed to destroy cancer cells, its tumor vasculature and tumor associated fibroblasts, expose neoantigens created by the tumor debris, inhibit the production of the extracellular matrix and enable immune cells to safely enter the tumor microenvironment. DeltaVax is a retrovector encoding the human GM-CSF gene, used for evoking T-cell proliferation, dendritic cell maturation and polarization of M1 macrophages. United States- and Philippine-based Phase I/II studies using DeltaRex-G for sarcoma, pancreatic cancer, and breast cancer led to its accelerated approval in the Philippines for all chemoresistant solid malignancies and subsequent USFDA approved Orphan Drug status for pancreatic cancer, soft tissue sarcoma and osteosarcoma. In 2009, DeltaRex-G received Fast Track designation for a pivotal Phase II/III trial for pancreatic cancer in the United States. The GeneVieve protocol added a second retrovector strategically to the DeltaRex-G

treatment that encoded a GM-CSF gene to examine the role localized GMCSF might play in further improving treatment outcomes and inducing long lasting anti-tumor immunity.

3.4.2 Methods

The patient population consisted of 16 adults with unresectable advanced or metastatic disease. All subjects had an ECOG score between 0 and 1, adequate hematological, kidney and hepatic function, and an estimated survival of 3 months or more. A chemistry panel and complete blood count were assessed weekly during treatment. DeltaRex-G was administered with escalating doses of DeltaVax, five patients at Dose Level I, four patients at Dose Level II, and seven patients at Dose Level III. All patients received a minimum of two cycles of treatment over an 8-week period. Toxicity was assessed prior to each infusion and subsequent treatment cycles using NCI CT-CAE version 3.0 criteria. A staging assessment was performed every 4 weeks with an FDG PET-CT scan. All images were performed and reviewed independently by the radiologists and RECIST v1.0 and International PET criteria were used to assess overall tumor response and progression-free survival.

3.4.3 Published results

No dose-limiting toxicities were observed at any of the three Dose Levels of DeltaVax, and no deaths that occurred were considered to be related to the treatment. None of the patients tested positive for vector neutralizing antibodies, replication-competent retrovirus in peripheral blood lymphocytes, antibodies to gp70 or vector integration into genomic DNA of peripheral blood lymphocytes. Using RECIST v1.0 criteria, three patients achieved a partial response, nine patients achieved stable disease, and two patients had progressive disease. The median progression free survival was 4.5, 9.0, and 13.0 months for Dose Levels I, II, and III respectively, and the median overall survival was 17, 13 and > 21 months for Dose Levels I, II, III respectively.

Histopathologic examination of patients' residual tumor tissues showed vector localization as well as GM-CSF transgene expression in necrotic tissue, displaying the accuracy in delivery of both treatments. Safety and tolerability are displayed by the lack of adverse reactions associated with the study drugs. The one-year survival rate of 86% in patients who received higher doses of DeltaVax suggests that the combination regimen of DeltaRex-G and Deltavax has significant anti-tumor activity in patients with chemoresistant solid malignancies and B-cell lymphoma. In addition, the substantial increase in progression free survival with each increased dosage of DeltaVax suggests a trend towards a positive dose–response relationship between the two treatments.

3.4.4 Conclusions/future directions

DeltaRex-G has displayed through numerous clinical trials its cytocidal effect on cancer cells. This effect introduces neoantigens from the tumor into the tumor microenvironment to be recognized by the immune system and targeted for destruction through T-cell mediation. Nevertheless, these cytotoxic immune responses may not be significant enough to overcome the suppressive signals from surrounding regulatory T-cells that may also be recruited to the tumor microenvironment. The addition of DeltaVax is hypothesized to heighten the development of dendritic cells and increase proliferation and activation of T-cells, thereby improving the potency of tumor-targeted DeltaRex-G. These activated T-cells can then go

on to recognize and destroy the newly introduced tumor neoantigens. This has the potential to further tumor regression and evoke long-lasting antitumor immunity.

This data therefore strongly suggests that the advancement of personalized cancer vaccination treatment has the potential to gain control of tumor growth and increase overall survival time in patients with advanced or malignant chemoresistant solid malignancies, as well as B-cell lymphomas.

4. Discussion/conclusion/summary

Targeted Immunotherapy has revolutionized the way scientists and physicians conceptualize their approaches to cancer treatment and cancer checkpoint controls. Mechanistic understanding of innate and adaptive mechanisms of immunity are considered important aspects of both physiological cancer surveillance and tumor eradication, as seen in immune checkpoint control and in precision blockade of cell cycle control elements. The low immunogenicity of cancer cells, as well as the tendency of advanced cancers to create an immunosuppressive tumor microenvironment presents a technical problem of precision tumor-targeted drug delivery for both immune checkpoint antibodies and cell cycle control elements, which form a rational basis for emerging treatments. The precision of monoclonal antibodies as checkpoint inhibitors targeting cancer cells has allowed research to advance in a direction that moves away from the untoward toxicities associated with chemotherapy towards treatments that enhance the naturally powerful cytotoxic responses of the immune system. The use of checkpoint inhibitors as cancer immunotherapy has been validated in 16 indications; however, immune checkpoint inhibition is still only considered appropriate for a specific subset of patients [58], and is often confounded by serious immune-related Adverse Events (imAEs). The significance and durability of response to treatment with checkpoint inhibitor therapy is generally dependent on tumor cells having a high mutational burden or microsatellite instability that creates an increased amount of neoantigens to be recognized and eliminated by the adaptive immune system [59]. Based on the documented physiological tumor-seeking behavior and demonstrated survival value of the tumor-targeted gene therapy vectors, DeltaRex-G and DeltaVax, in treating advanced metastatic cancers, the successful adaptation of bioactive gene-targeting biotechnologies to (i) target FDA-approved off-the shelf checkpoint monoclonal antibodies to tumors, and/or (ii) recombinant "tumor-targeted" adaptor proteins have been developed, in anticipation of precisely targeting immune checkpoint inhibitors and immunostimulatory cytokines against tumors to improve clinical outcomes.

Another strategic approach is enhancing the anti-tumor properties of innate immunity. The innate immune system is also regulated by its own activating and inhibitory pathways that can be investigated as future targets for NK cell-based immunotherapy. One important characteristic to consider when making the case for focusing on boosting innate immunity is the fact that innate immune cells play a major role in immunosurveillance, acting as the first line of defense. Engaging the innate immune system is a necessary prerequisite for antigen-specific T-cells to respond, although innate immune cells such as NK cells do not require activation of T-cells to kill tumor cells [58]. NK cell activation occurs through their direct interaction with target cells, bypassing the need for antigen presentation and processing. Innate immunity is always activated prior to adaptive immunity, however, once activated, adaptive immunity has the advantage of higher specificity and lower probability of self-harm.

In recent years, the human Cyclin G1 (*CCNG1*) gene was established as a central executive element of a Commanding Cyclin G1/Cdk/Mdm2/p53 Axis: representing

a strategic locus for restoring cell cycle checkpoint control through precision gene transfer. With the development of the first tumor-targeted cancer gene therapy, DeltaRex-G [60], it became possible for patients to (i) benefit clinically, (ii) enjoy good quality of life and (iii) survive appreciably longer without experiencing the debilitating toxicities of chemotherapy. The tumor-targeted DeltaRex-G vector consists of bioactive nanoparticles displaying a high-affinity targeting motif on its surface for "pathotropic" (lesion-seeking) targeting by binding to abnormal signature (SIG) proteins found abundantly in invading tumors, and then delivering a cytocidal genetic payload, a CCNG1 cell cycle checkpoint inhibitor gene, into rapidly dividing cancer cells, tumor associated microvasculature and tumor-associated fibroblasts, without collateral damage to normal cells and non-target organs. The observed reduction in tumor matrix production and tumor destruction paved the way for enhanced innate immune cell entry into the tumor microenvironment. The enhanced immune cells consist of cytotoxic T cells, NK cells, and dendritic cells for cell recognition, destruction and autoimmunization, as well as regulatory immune cells to prevent exaggerated immune responses that cause cytokine release syndrome or cytokine storm.

Hence, DeltaRex-G eradicates cancer cells without causing immune-mediated adverse events, an unwanted complication of immune checkpoint inhibitors such as ipilimumab, nivolumab, pembrolizumab, atezolizumab, etc. Conceivably, DeltaRex-G could also be used in combination with reduced doses of immune checkpoint inhibitors to minimize off-target toxicity (imAEs) and maximize anticancer efficacy.

A second tumor-targeted retrovector, DeltaVax, displaying the same high-affinity tumor-targeting motif as DeltaRex-G, but this immuno-vector—encoding both the GM-CSF gene and the pro-drug regulated HSV-tk gene, and allowing for personalized "pulsed" *in situ* vaccinations—demonstrated promising results in a small Phase I/II study conducted in Manila, Philippines with considerable clinical benefit: good quality of life and an 86% one year survival rate in patients with advanced chemotherapy-resistant Stage 4 malignancies and a uniformly poor prognosis.

In the era of precision medicine, with tumor-targeted cancer gene therapy and immunotherapy coming of age, these recent advances bring great optimism to the medical and scientific communities around the world and the patients that they serve.

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Conflict of interest

Drs. Gordon and Hall are co-inventors of the targeted gene delivery system represented by DeltaRex-G and DeltaVax which was originally developed at the University of Southern California Keck School of Medicine, and are co-founders of Delta Next-Gene, LLC. Dr. Gordon is founder and president of the Aveni Foundation, a 501c3 public charity. NLA, TTK, DAB and SPC have no competing interest.

Ethics approval and consent to participate

The clinical protocol was approved by the USFDA, the Western IRB, and the Institutional Biosafety Committee. A written informed consent was obtained from each patient prior to treatment with an investigational agent.

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