

Review

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

Paul Stendahl G. Dy¹, Sant P. Chawla¹, Frederick L. Hall², Erlinda M. Gordon^{*1,2}

1. Cancer Center of Southern California, Santa Monica CA

2. Delta Next-Gen, LLC, Santa Monica CA

*Corresponding author: Erlinda M. Gordon, MD, Director, Biological and Immunological Therapies, Cancer Center of Southern California/Sarcoma Oncology Center, 2811 Wilshire Blvd., Suite 414, Santa Monica CA 90403, Tel: 310-552-9999; E-mail: egordon@sarcomaoncology.com

Received: August 25, 2018; Accepted: September 07, 2018; Published: September 12, 2018

Abstract

Background:

The quest for seeking out and eradicating remote, occult, and metastatic cancers remains elusive. More advanced precision-targeted therapies are needed; DeltaRex-G, a tumor-targeted retrovector encoding a dominant negative human cyclin G1 inhibitor is a perceptive addition.

Method:

The researchers reviewed and summarized published literature on immune cell trafficking within the microenvironment of DeltaRex-G-treated tumors.

Results:

The studies reveal a telltale cadre of immune cells composed of killer T cells, helper T cells, natural killer cells, dendritic cells, B cells, and macrophages/monocytes infiltrating into various residual tumor nodules alongside tumor destruction.

Conclusion:

DeltaRex-G enables the 1) entry of immune cells and immune checkpoint inhibitors into the tumor microenvironment by destroying the extracellular matrix-producing stromal fibroblasts and 2) preservation of a patient's innate tumor surveillance function by sparing the bone marrow and the immune system from collateral damage. Hence, DeltaRex-G can be synergized with known immunotherapy agents to manage metastatic disease.

Background

The tragedy of metastatic cancer is immense principally because of its grim prognosis [1]. Despite numerous approved treatment regimens presently in place, more novel therapies need to be added to the oncologists' arsenal. "Pathotropic (disease-seeking) targeting" has recently shown great promise in the clinic. Since 2003, DeltaRex-G (former name: Mx-dnG1, Rexin G) - a tumor targeted retrovector - has been improving overall survival in intractable cancers such as pancreatic cancer, malignant melanoma, bone and soft tissue sarcoma, breast cancer, renal cell carcinoma, and B-cell lymphoma [2-5].

By inhibiting the "human cyclin G1 (CCNG1)" cell cycle control pathway, DeltaRex-G arrests the proliferative

cycle in G1 phase and, consequently, causes apoptosis of tumor cells [6]. The elegance of DeltaRex-G as a promising cancer gene therapy lies in its tumor-targeted "nanoparticles" that selectively seek out and accumulate in the tumor microenvironment (TME) by binding to abnormally exposed collagenous proteins caused by the invading tumor [3,4,6].

To date, more research needs to highlight the mechanisms of DeltaRex-G therapy - particularly its ability to increase immune cell entry and its synergism with known cancer immunotherapy agents. This review summarizes the published literature on immune cell trafficking in the tumor microenvironment (TME) of excised tumors

following DeltaRex-G therapy.

We extend our working hypothesis that, in addition to killing the cancer cells and associated tumor vasculature, DeltaRex-G would enable 1) the entry of immune cells and immune checkpoint inhibitors into the tumor microenvironment by destroying the extracellular matrix-producing stromal fibroblasts and 2) the preservation of a patient's innate tumor surveillance function by sparing the bone marrow and the immune system from collateral damage. Hence, DeltaRex-G can be combined synergistically with immunotherapy, such that identification of strategic combinatorial regimens would advance the clinical utility of DeltaRex-G in the management of metastatic disease.

Methods

A review of published literature across various scientific journals which have published and/or discussed DeltaRex-G as a novel precision targeted gene therapy for cancer was conducted. The types of human cancers included metastatic pancreatic cancer, pancreatic B-cell lymphoma, malignant melanoma, breast adenocarcinoma, non-small cell lung carcinoma, and osteosarcoma. The

immunohistochemical staining characteristics of excised tumors from DeltaRex-G-treated patients were extracted from the published sources and summarized [2-8].

Results

Table 1 enumerates the tumor-infiltrating lymphocytes seen in excised tumors of DeltaRex-G-treated cancer patients. Visual examples from 2 patients with metastatic pancreatic cancer show a preponderance of immune cells interspersed with tumor destruction (Figures 1-3) [2,7].

In these cytological characterizations, anti-tumor immune cells must be differentiated from pro-tumor immune cells. Agents proven to be on the anti-tumor arm of the immune system include dendritic cells, natural killer cells, helper T cells, and killer T cells [7,8]. The killer T cells are invaluable in controlling tumor growth and metastasis. These lymphocytes - with the aid of antigen presenting cells (e.g., dendritic cells) - are recruited when tumor cells first appear. The majority of the helper T cells work in coordination with the killer T cells. Natural killer cells act as support for killer T cells by attacking the tumor cells that are rendered undetectable to the latter by the lack of major histocompatibility complexes [8]. Regulatory T

Table 1. Immune cell trafficking in the TME of DeltaRex-G-treated tumors.

Excised Cancer Type	Immune Cell Type	Ref
Pancreatic adenocarcinoma metastatic to liver	Anti-tumor: <ul style="list-style-type: none"> ● Killer T cells (CD8+) ● Natural killer cells (CD56+) ● Helper T cells (CD4+) ● Dendritic cells (CD35+) Possibly pro-tumor: <ul style="list-style-type: none"> ● B cells (CD20+) ● Leucocyte common antigen (CD45+) 	4
Pancreatic adenocarcinoma metastatic to liver	Anti-tumor: <ul style="list-style-type: none"> ● Killer T cells (CD8+) ● Helper T cells (CD4+) Possibly pro-tumor: <ul style="list-style-type: none"> ● Leucocyte common antigen (CD45+) 	7
Malignant melanoma metastatic to inguinal lymph node	Anti-tumor: <ul style="list-style-type: none"> ● Killer T cells (CD8+) ● Helper T cells (CD4+) ● Dendritic cells (CD35+) Possibly pro-tumor: <ul style="list-style-type: none"> ● Leucocyte common antigen (CD45+) 	4

Colorectal cancer metastatic to lung	Anti-tumor: <ul style="list-style-type: none"> ● Killer T cells (CD8+) ● Helper T cells (CD4+) Possibly pro-tumor: <ul style="list-style-type: none"> ● B cells (CD20+) 	5
Pancreatic B-cell lymphoma metastatic to liver, cervical lymph node	Anti-tumor: <ul style="list-style-type: none"> ● Killer T cells (CD8+) 	5
Breast ductal adenocarcinoma, recurrent	Anti-tumor: <ul style="list-style-type: none"> ● Killer T cells (CD8+) ● Helper T cells (CD4+) ● Dendritic cells (CD35+) ● M1 macrophages (CD68+) Possibly pro-tumor: <ul style="list-style-type: none"> ● B cells (CD20+) ● Macrophages (CD68+) 	8
Non-small cell lung carcinoma metastatic to adrenal gland	Anti-tumor: <ul style="list-style-type: none"> ● Killer T cells (CD8+) ● Natural killer cells (CD56+) ● Helper T cells (CD4+) ● Dendritic cells (CD35+) Possibly pro-tumor: <ul style="list-style-type: none"> ● B cells (CD20+) ● Leucocyte common antigen (CD45+) 	8

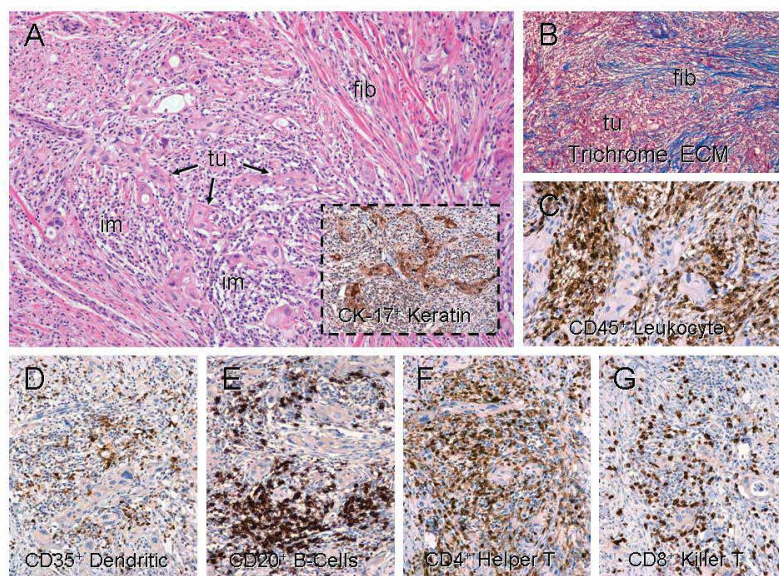


Figure 1. Immunohistochemical characterization of tumor-infiltrating lymphocytes in tumor. **a** Tumor nodule with a cyokeratin-17 immunostain; H&E. **b** Collagenous material (bluish material); trichrome stain. **c** Cells expressing the leukocyte common antigen (reddish material). Immunohistochemistry for **d** CD35+ dendritic cells, **e** CD20+ B cells, **f** CD4+ helper T cells, and **g** CD8+ killer T cells. ECM, extracellular matrix; fib, fibrosis; im, immune cells; tu, tumor [2].

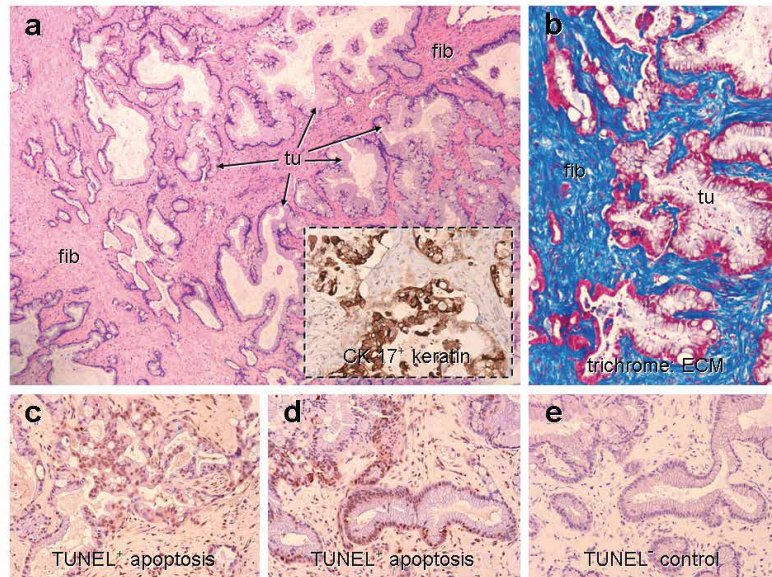


Figure 2. Histological section of excised liver tumor. **a** This shows preponderance of fibrosis (fib) with moderately differentiated epithelioid tumor cells (tu) arrayed in columnar/ductal structures, seen in various stages of degeneration; H&E stain, pancreatic cancer cells marked by a cytokeratin-17 immunostain (inset) **b** Abundant fibrosis is observed throughout the tumor nodule, as shown by Masson's trichrome stain for ECM **c, d, e** Extensive apoptosis of the tumor vasculature and tumor cells, stromal fibroblasts, as well as visible karyorrhexis are evident along the borders of the glandular structures [7].

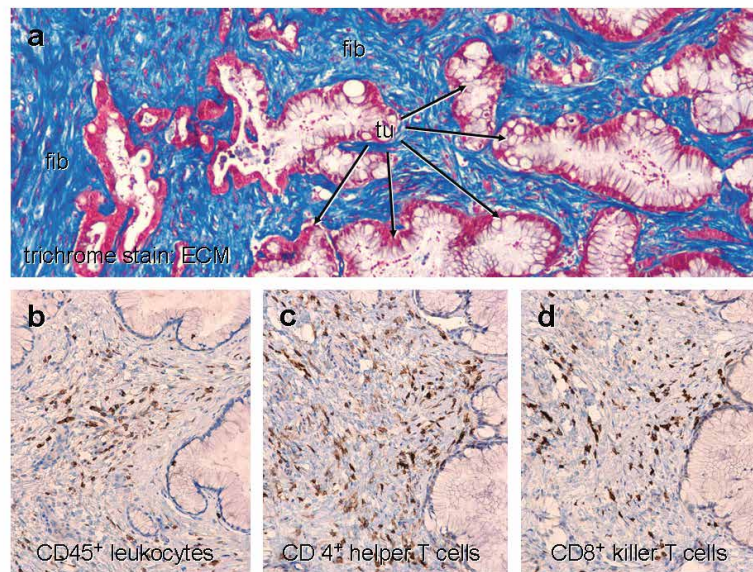


Figure 3. Further characterization of the immune infiltrate in the nodule. **a** The immune cells are interspersed within the reactive/repairative fibrosis (fib) that surrounds the tumor cells (tu) of the excised nodule with the reddish material being keratin and the bluish material, ECM; Masson's trichrome stain. This characterization reveals that the cadre of recruited immune cells, collectively marked by the **b** CD45 common leucocyte antigen, contains both **c** CD4+ helper T-cells and **d** CD8+ killer T-cells; the latter of which are selectively cytotoxic, adaptive components of cell-mediated tumor immunity [7].

cells, “tumor-associated macrophages (TAMs)”, and B cells can abet the tumor cells and protect them against the immune system. Regulatory T cells can impede antigen presentation and killer T cell action. M1-type TAMs can elicit anti-tumor inflammation, but they can be overpowered by M2-type TAMs that promote tumor pathogenicity; hence, the potential pro-tumor attribute of macrophages. B cells can also help recognize tumor cells for other immune cells to target, but a subtype (i.e., regulatory B cells), can foster tumor growth by producing “neo-angiogenesis and immune suppressive cytokines” [1,9].

Discussion

We consistently observed that DeltaRex-G induces apoptosis in tumor cells, proliferative vasculature, and ECM producing stromal fibroblasts. Moreover, the direct cytotoxic activity observed within the DeltaRex-G treated tumors was generally associated with a robust immune response that involves both cell-mediated and humoral immunity juxtaposed with overt apoptosis and necrosis of tumor cells. This underscores the advantageous pleiotropic effects of DeltaRex-G: killing the tumor cells, and conceivably, promoting cancer immunization *in situ* [4,7].

Enhanced immune cell trafficking in excised tumors of DeltaRex-G-treated patients suggest an underlying immunologic mechanism for the noted improvements in survival reported in these patients [2,3]. In fact, three patients, each with a different type of intractable cancer (e.g., metastatic pancreatic cancer, B-cell lymphoma, and metastatic osteosarcoma), are still alive 9 years following courses of CCNG1 inhibitor therapy; to date, their chemo-resistant cancers have not recurred nor did they require additional cancer treatments [3]. However, we also consider the possibility of inadvertently recruiting pro-tumor immune cells that might foster tumor growth and metastasis, such as certain tumor-associated macrophages and B cells [1,9].

Immunotherapy

The ideal of prompting, and even enhancing, the body’s own immune surveillance system in the clinical management of cancer is in complete accord with the stated goals of precision medicine. The cytotoxic mechanisms of action of tumor-targeted DeltaRex-G, which appears to recruit the immune system into the TME, runs parallel to the principle of immunotherapy. The latter approach has achieved some success in the past years with checkpoint inhibitors/blockers: including the anti-Cytotoxic T-Lymphocyte-Associated protein 4 (CTLA-4; ipilimumab) for metastatic melanoma; the anti-Programmed Cell Death protein 1 (PD-1; nivolumab, pembrolizumab, atezolizumab) for non-small cell lung cancer; and so-called adoptive T cell therapies, using customized genetically engineered antigen-specific T cell clones.

Other types of investigational immunotherapies include cancer vaccines, oncolytic viruses, and monoclonal antibodies [10]. Together, the emerging diversity of immunomodulatory treatment options, each with very distinct mechanisms of action, represents a promising approach in terms of new combinatorial therapies.

Delta-Rex G and Immunotherapy

To optimize cancer immunotherapy, the innate and adaptive components of the immune system must work together [10,11]. Delta-Rex G can conceivably synergize with different types of immunotherapy to enhance the beneficial effects and reduce immune-related toxicities. For example, anti-PD-1 inhibitors work well with cancers that already have pre-existing killer T cell infiltrates; the anti-PD1 inhibitors can be combined with treatment that can increase anti-tumor T cells [10]. Targeted drug therapy with immunomodulators like lenalidomide can be combined with natural killer T cell therapy to enhance the latter’s ability to kill the tumor cells [11].

Targeted gene therapies can also be used to modulate the pro-tumor effects of specific immune cells, such as the regulatory B cells and macrophages (TAMs). Reximmune-C, a tumor-targeted retrovector encoding the human granulocyte / macrophage colony-stimulating factor (GM-CSF) gene, was reported to extend the overall survival of cancer patients treated with DeltaRex-G followed by Reximmune-C in an attempt to effectuate a longer lasting anti-tumor immunity [5].

Limitations and Future Studies

DeltaRex-G, in combination with strategic cancer immunotherapy drugs, is likely to evoke a more robust anti-tumor immunity, as well as greater antitumor response. However, the clinical benefit of these combinatorial therapies needs further evaluation.

Studies are planned to further evaluate immune cell trafficking in tumor compartments before, during, and after treatment with DeltaRex-G (CCNG1 inhibitor) therapy. The new immunologic findings will be correlated with treatment outcome parameters (i.e., tumor response, progression-free survival and overall survival), as well as quantitative immunological and histological data.

Effectuating and monitoring cytotoxic T cell infiltration with tumor-targeted DeltaRex-G infusions sets the stage for combinatorial regimens using DeltaRex-G with targeted immunotherapy agents. By pursuing this avenue of precision medicine in cancer therapy, both the treatment and the prognosis of metastatic cancer are likely to be improved, which would bring us closer to an achievable cancer cure.

Abbreviations

CCNG1: Cell cycle human cyclin G1; CTLA4: Cyto-

toxic T-lymphocyte-associated protein 4; GM-CSF: Granulocyte macrophage colony stimulating factor; TAMs: Tumor-associated macrophages; TME: Tumor microenvironment; PD-1: Programmed cell death protein 1

Acknowledgements

The authors gratefully acknowledge previous funding from the National Science Foundation, National Institutes of Health, NATO Scientific Exchange Program, American Heart Association, Whittier Family Foundation, and the FDA Orphan Drug Program in support of this pioneering biomedical research.

Funding

Funded in part by the Sarcoma Oncology Center and in part by Delta Next-Gen, LLC and The Cancer Center of Southern California

Ethics approval and consent to participate: Not applicable

Consent for publication: Not applicable

Availability of data and material

The authors understand that the materials included in the manuscript, including all relevant raw data, will be made freely available to any researchers who wish to use this for non-commercial purposes, while preserving any necessary confidentiality and anonymity. The data in this short communication are available in References 2-8.

Conflict of interest

EMG and FLH are co-inventors of DeltaRex-G targeted gene delivery system, which was developed at the University of Southern California Keck School of Medicine, and are co-founders of Delta Next-Gen, LLC. PSD and SPC have no competing interest.

Authors' contributions

PSD reviewed the published literature, analyzed the data, wrote the manuscript, table and references, and

reviewed the final manuscript. SPC and FLH reviewed the published literature, analyzed the data using DeltaRex-G, reviewed and edited the final manuscript. EMG reviewed the published literature, analyzed the data, proposed the hypothesis, re-wrote sections of the manuscript, reviewed and edited the final manuscript. All authors approve the submission of this manuscript for publication.

References

1. Kitamura T, Qian B-Z, Pollard JW. Immune cell promotion of metastasis. *Nat Rev Immunol.* 2015;15(2):73–86.
2. Chawla S, Chua V, Fernandez L, et al. Advanced Phase I/II Studies of Targeted Gene Delivery In Vivo: Intravenous REXIN-G for Gemcitabine-resistant Metastatic Pancreatic Cancer. *Molecular Therapy.* 2010;18(2):435-441.
3. Kim S, Federman N, Gordon E, et al. REXIN-G®, a tumor-targeted retrovector for malignant peripheral nerve sheath tumor: A case report. *Molecular and Clinical Oncology.* 2017; 6(6):861-865.
4. Gordon E, Lopez F, Cornelio G, et al. Pathotropic nanoparticles for cancer gene therapy REXIN-G™ IV: Three-year clinical experience. *Int J Oncol.* 2006; 29:1053-1064.
5. Ignacio J, Bruckner H, Manalo R, et al. Tumor-targeted cancer vaccination (GeneVieve Protocol): A phase I/II study of intravenous REXIN-G and REXIMMUNE-C for chemotherapy-resistant cancers. *J Clin Oncol.* 2011; 29(15_suppl):2589.
6. Gordon EM, Ravicz JR, Liu S, et al. Cell cycle checkpoint control: The cyclin G1/Mdm2/p53 axis emerges as a strategic target for broad-spectrum cancer gene therapy - A review of molecular mechanisms for oncologists. *Molec Clin Oncol.* 2018;9:115-134.
7. Hall FL, Levy JP, Reed RA, et al. Pathotropic targeting advances clinical oncology: Tumor-targeted localization of therapeutic gene delivery. *Oncol Rep.* 2010; 24:829-833.
8. Gordon E, Chan M, Geraldino N, et al. Le morte du tumour: Histological features of tumor destruction in chemo-resistant cancers following intravenous infusions of pathotropic nanoparticles bearing therapeutic genes. *Int J Oncol.* 2007;30:1297-1307.
9. Caruana I, Simula L, Locatelli F, et al. T lymphocytes against solid malignancies: winning ways to defeat tumours. *Cell Stress.* 2018;2(8):200-212.
10. Spranger S, Gajewski T. Rational combinations of immunotherapeutics that target discrete pathways. *J for ImmunoTherapy of Cancer.* 2013;1(1):16.
11. Jungkuntz-Stier I, Zekl M, Stühmer T, et al. Modulation of natural killer cell effector functions through lenalidomide/dasatinib and their combined effects against multiple myeloma cells. *Leukemia & Lymphoma.* 2013;55(1):168-176.

To cite this article: Paul Stendahl G, Dy, Sant P, Chawla, Frederick L, Hall, Erlinda M, Gordon. Immune cell trafficking in the tumor microenvironment of human cyclin G1 (CCNG1) inhibitor-treated tumors. *British Journal of Cancer Research.* 2018; 1:4. doi: 10.31488/bjcr.117

© Stendahl DyPG, et al. 2018.