


Editorial

# Mobilizing Toxins for Cancer Treatment: Historical Perspectives and Current Strategies

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The level of complexity in a disease like cancer presents a number of challenges for effective treatment development, which require significant innovation to overcome. Enthusiasm for immunotherapies and other types of biotherapeutics has grown substantially over the past decade, as additional insight into the interplay between tumors and the immune system has allowed for a departure from harsher conventional systemic treatments. However, amidst these impressive advances, these therapies may still fall short for many patients. Faced with this dilemma, more biotherapeutic options continue to be researched as potential primary or adjuvant treatments.

For millennia, poisonous compounds have been used for medicinal purposes such as mild pain relief or numbing during surgery. Even in the modern age, plant and animal toxin-derivatives continue to be widely used as treatments for a variety of ailments. The anticoagulants tyrofabin and hirudin, for example, originate from venom of the African saw-scaled viper and leech secretions, respectively [1]. Even pathogenic bacteria typically considered harmful to healthy tissue may prove to be clinically useful as studies have shown that toxins produced by these organisms can be manipulated to target aberrant cells in a tissue- or cell-specific manner [2–5].

In this Special Issue, we explore how toxins may be used as powerful treatments against certain cancers. The compiled articles cover how naturally-derived poisons can be utilized for cancer therapy on multiple levels, from interrogating cytotoxic pathways in different cell types, to exploiting toxic derivatives for pain relief in patients suffering from radiation sickness [3,5]. The Special Issue presented this month helps to expound upon this field of research and demonstrate the potential for its clinical applicability.

One of the first uses of toxins as cancer treatments dates back to the early twentieth century, most notably by William Coley, a bone surgeon who discovered that a combination of heat-killed and systemically administered bacteria could shrink osteosarcomas [6,7]. The inception of cancer immunotherapy can arguably be traced back to the innovation of “Coley’s Toxins,” which initiated queries into how a patient’s immune system can be triggered to kill cancer cells [2]. Immunoediting, a prominent idea in the field of immunotherapy [8], asserts that while the immune system is at first able to recognize and kill portions of cancer cell populations, the cancer gradually develops mutations that permit evasion of immune detection, allowing for tumor growth and eventual metastasis [2]. Over the past several decades, clinical strategies to overcome stagnancy in cytotoxic T cell or NK cell responses include utilizing immune checkpoint inhibitors, such as PD-1/PD-L1 or CTLA-4 blockade [9–12], or direct infusion of cytokines like IFN $\alpha$  [13,14]. Moreover, vaccines against neoantigens [15] and known tumor-associated antigens, could prove useful for patients with genetic predispositions to cancer. Currently, there is a phase II clinical trial investigating the ability of a mucin 1 (MUC1) vaccine to prevent adenoma recurrence in patients at high-risk of colorectal cancer [16]. Oncolytic virotherapy, like the FDA-approved talimogene laherparepvec (“T-VEC”) [17,18], is yet another instance of a

therapeutic derived from bioengineering. Remarkably, this kind of virotherapy works to reshape and adapt the tumor microenvironment (TME) to boost immune infiltration.

The mechanism of action for such biotherapeutics must be well understood for effective employment of the treatment, as one study considers. Shiga toxins (Stxs) produced by *Escherichia coli* and *Shigella dysenteriae* 1 pathogenic bacteria bind to the cell surface receptor glycosphingolipid globotriaosylceramide (Gb3) [19] and induce apoptosis by inhibiting protein synthesis [3]. Gb3 is highly upregulated in Burkitt lymphoma (BL) cells [20], and Stx/verotoxins, VT-1 and VT-2, have been used in several preclinical studies, albeit with little success due to abundant cytotoxicity and poor understanding of verotoxin-induced apoptosis [21]. Detailed in one paper, treating BL cells with VT-1/Stx1 consistently induces the endoplasmic reticulum (ER) stress response by activating ER stress sensors, IRE1 and ATF6, as well as increasing expression of the transcription factor C/REB homologous protein (CHOP) that normally signals for programmed cell death. The role of VT-1 in cell death is noted to be cell-specific, and in fact may shield certain tumor cells from death instead of inducing apoptosis. ER stress enhances VT-1-induced apoptosis through CHOP in BL2 cells, but not in Ramos cells [3]. Strikingly, VT-1-induced ER stress triggers ER-phagy that in turn restrains apoptosis in Ramos cells.

*Escherichia coli* protein toxin, cytotoxic necrotizing factor 1 (CNF1), acts as an effective anti-neoplastic in glioma mouse models, reducing tumor volume and increasing survival, all while preserving the functional properties of the surrounding neurons [22,23]. As one paper acknowledges, therapies against glioma cells must be able to cross the blood-brain barrier (BBB), otherwise, treatment would have to be directly injected into the brain [4]. To circumvent invasive cranial injections, CNF1 was reengineered with an N-terminal BBB-crossing tag. Not only does this BBB-CNF1 variant, referred to as the An2-CNF1-H8 variant, show comparable activity to its wild-type (WT) counterpart, but it is also able to be purified in native conditions. This variant also exerts cell growth arrest of U87MG GBM cells in a similar fashion to unmodified WT-CNF1 and upregulates pro-apoptotic protein Bax expression. Experiments performed on endothelial cells demonstrate that the An2-CNF1-H8 variant is able to enter cells and perform its intended functions, as indicated by equivalent actin architecture changes to that of the WT. Intravenous administration of the An2-CNF1-H8 variant upregulates spinophilin in the mouse hippocampus, suggesting BBB bypass. Altogether, these results demonstrate that the An2-CNF1-H8 variant is likely able to cross the BBB to induce cell death in GBM cells [4] and may be translated to future clinical studies.

Similar to clinically-approved CD3-based bispecifics, some immunotoxins utilize antibody-like specificity to recognize tumor antigens, while also possessing a toxic domain that releases a toxin into the target cell following internalization [24,25]. One study explores how immunotoxin efficacy could be improved by modulating the intracellular trafficking of the toxin [26]. The inclusion of a furin cleavage site allows immunoconjugates derived from RNase T1 and the fungal ribotoxin  $\alpha$ -sarcin (scFvA33furT1 and IMTXA33fur $\alpha$ S, respectively) to be purified with optimized properties for colorectal tumor treatment. It is also noted that the two immunotoxins are trafficked in different pathways after endocytosis. After binding to their target GPA33 on the surface of W1222 colorectal cancer cells, IMTXA33fur $\alpha$ S goes through the endosome-Golgi-apparatus network, and scFvA33furT1 appears distributed between the lysosomes and the Golgi-apparatus. The differences in trafficking pathways between the two immunoconjugates align with what is observed from their original constructs [27]. In vitro functional characterization of these variants demonstrates enhanced antitumor efficiency due to increased ability to release their toxic domain into the cytosol, as well as high thermostability and target specificity.

Aside from direct applications as cancer treatments, toxins could be used to mitigate pain directly caused by tumor pressure, or neuropathic pain as a side effect of radiation or surgery in cancer patients [5]. Many clinical studies [28–38] have investigated the use of botulinum neurotoxins (BoNT) as potent systemic analgesics, as these toxins block acetylcholine release from the neuromuscular junction or inhibit neurotransmitters at both peripheral and central sensory levels [39–42]. Additionally, some sources claim that spiking certain cancer cell lines with BoNT slows growth and mitosis, as well

as enhances apoptosis [43]. Studies of pain induced by radiation and/or surgery suggest that the local injection of BoNT improves neuropathic pain and local muscle spasm in the direct vicinity of the site of surgery and/or radiation. However, this type of pain-management therapy requires blinded and placebo-controlled studies to confirm its efficacy [5]. The results from various studies investigating the use of BoNT as an anti-tumor therapeutic also show promise. In several in vivo experiments, direct injection of BoNT into various malignant tumors demonstrated cellular apoptosis and reduction of tumor size [44–46]. Adding BoNT (Type A) to a diverse range of cancer cell cultures showed slowed cell growth, as well as induction of apoptosis and reduction of mitotic activity [47–52].

Although some cancers have been treated with relative success in the past twenty years, there still remains a paucity of options for patients with difficult to treat, relapsing, or rare cancers. Indeed, cancer is surpassing cardiovascular disease to become the leading cause of death in many populations around the world. This Special Issue presents impactful research that explores the use of toxins as feasible and pertinent cancer therapies which some day may be the solution for so many suffering patients.

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## References

- Poison as Medicine|American Museum of Natural History. Available online: <https://www.amnh.org/explore/news-blogs/on-exhibit-posts/the-power-of-poison-poison-as-medicine> (accessed on 15 June 2020).
- Carlson, R.D.; Flickinger, J.C.; Snook, A.E. Talkin' toxins: From coleys' to modern cancer immunotherapy. *Toxins* **2020**, *12*, 241. [CrossRef] [PubMed]
- Debernardi, J.; Pioche-Durieu, C.; Cam, E.L.; Wiels, J.; Robert, A. Verotoxin-1-Induced ER Stress Triggers Apoptotic or Survival Pathways in Burkitt Lymphoma Cells. *Toxins* **2020**, *12*, 316. [CrossRef] [PubMed]
- Colarusso, A.; Maroccia, Z.; Parrilli, E.; Germinario, E.A.P.; Fortuna, A.; Loizzo, S.; Ricceri, L.; Tutino, M.L.; Fiorentini, C.; Fabbri, A. Cnf1 Variants Endowed with the Ability to Cross the Blood-Brain Barrier: A New Potential Therapeutic Strategy for Glioblastoma. *Toxins* **2020**, *12*, 291. [CrossRef] [PubMed]
- Mittal, S.O.; Jabbari, B. Botulinum Neurotoxins and Cancer-A Review of the Literature. *Toxins* **2020**, *12*, 32. [CrossRef] [PubMed]
- Coley, W.B. Contribution to the Knowledge of Sarcoma. *Ann. Surg.* **1891**, *14*, 199–220. [CrossRef] [PubMed]
- McCarthy, E.F. The toxins of William B. Coley and the treatment of bone and soft-tissue sarcomas. *Iowa Orthop. J.* **2006**, *26*, 154–158.
- Schreiber, R.D.; Old, L.J.; Smyth, M.J. Cancer immunoediting: Integrating immunity's roles in cancer suppression and promotion. *Science* **2011**, *331*, 1565–1570. [CrossRef]
- Topalian, S.L.; Hodi, F.S.; Brahmer, J.R.; Gettinger, S.N.; Smith, D.C.; McDermott, D.F.; Powderly, J.D.; Carvajal, R.D.; Sosman, J.A.; Atkins, M.B.; et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N. Engl. J. Med.* **2012**, *366*, 2443–2454. [CrossRef]
- Garon, E.B.; Rizvi, N.A.; Hui, R.; Leighl, N.; Balmanoukian, A.S.; Eder, J.P.; Patnaik, A.; Aggarwal, C.; Gubens, M.; Horn, L.; et al. KEYNOTE-001 Investigators Pembrolizumab for the treatment of non-small-cell lung cancer. *N. Engl. J. Med.* **2015**, *372*, 2018–2028. [CrossRef]
- Hodi, F.S.; O'Day, S.J.; McDermott, D.F.; Weber, R.W.; Sosman, J.A.; Haanen, J.B.; Gonzalez, R.; Robert, C.; Schadendorf, D.; Hassel, J.C.; et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N. Engl. J. Med.* **2010**, *363*, 711–723. [CrossRef]

12. Brahmer, J.R.; Tykodi, S.S.; Chow, L.Q.M.; Hwu, W.-J.; Topalian, S.L.; Hwu, P.; Drake, C.G.; Camacho, L.H.; Kauh, J.; Odunsi, K.; et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N. Engl. J. Med.* **2012**, *366*, 2455–2465. [[CrossRef](#)] [[PubMed](#)]
13. Howie, J.W. Experiments with interferon in man: A report to the medical research council from the scientific committee on interferon. *Lancet* **1965**, *1*, 505–506. [[PubMed](#)]
14. Müller, U.; Steinhoff, U.; Reis, L.F.; Hemmi, S.; Pavlovic, J.; Zinkernagel, R.M.; Aguet, M. Functional role of type I and type II interferons in antiviral defense. *Science* **1994**, *264*, 1918–1921. [[CrossRef](#)]
15. Jiang, T.; Shi, T.; Zhang, H.; Hu, J.; Song, Y.; Wei, J.; Ren, S.; Zhou, C. Tumor neoantigens: From basic research to clinical applications. *J. Hematol. Oncol.* **2019**, *12*, 93. [[CrossRef](#)]
16. Kimura, T.; McKolanis, J.R.; Dzubinski, L.A.; Islam, K.; Potter, D.M.; Salazar, A.M.; Schoen, R.E.; Finn, O.J. MUC1 vaccine for individuals with advanced adenoma of the colon: A cancer immunoprevention feasibility study. *Cancer Prev. Res.* **2013**, *6*, 18–26. [[CrossRef](#)]
17. Lawler, S.E.; Speranza, M.-C.; Cho, C.-F.; Chiocca, E.A. Oncolytic viruses in cancer treatment: A review. *JAMA Oncol.* **2017**, *3*, 841–849. [[CrossRef](#)] [[PubMed](#)]
18. Andtbacka, R.H.I.; Kaufman, H.L.; Collichio, F.; Amatruda, T.; Senzer, N.; Chesney, J.; Delman, K.A.; Spitler, L.E.; Puzanov, I.; Agarwala, S.S.; et al. Talimogene laherparepvec improves durable response rate in patients with advanced melanoma. *J. Clin. Oncol.* **2015**, *33*, 2780–2788. [[CrossRef](#)] [[PubMed](#)]
19. Johannes, L. Shiga Toxin-A Model for Glycolipid-Dependent and Lectin-Driven Endocytosis. *Toxins* **2017**, *9*, 340. [[CrossRef](#)] [[PubMed](#)]
20. Nudelman, E.; Kannagi, R.; Hakomori, S.; Parsons, M.; Lipinski, M.; Wiels, J.; Fellous, M.; Tursz, T. A glycolipid antigen associated with Burkitt lymphoma defined by a monoclonal antibody. *Science* **1983**, *220*, 509–511. [[CrossRef](#)] [[PubMed](#)]
21. Engedal, N.; Skotland, T.; Torgersen, M.L.; Sandvig, K. Shiga toxin and its use in targeted cancer therapy and imaging. *Microb. Biotechnol.* **2011**, *4*, 32–46. [[CrossRef](#)] [[PubMed](#)]
22. Fabbri, A.; Travaglione, S.; Rosadi, F.; Ballan, G.; Maroccia, Z.; Giambenedetti, M.; Guidotti, M.; Ødum, N.; Krejsgaard, T.; Fiorentini, C. The Escherichia coli protein toxin cytotoxic necrotizing factor 1 induces epithelial mesenchymal transition. *Cell Microbiol.* **2020**, *22*, e13138. [[CrossRef](#)] [[PubMed](#)]
23. Vannini, E.; Olimpico, F.; Middei, S.; Ammassari-Teule, M.; de Graaf, E.L.; McDonnell, L.; Schmidt, G.; Fabbri, A.; Fiorentini, C.; Baroncelli, L.; et al. Electrophysiology of glioma: A Rho GTPase-activating protein reduces tumor growth and spares neuron structure and function. *Neuro. Oncol.* **2016**, *18*, 1634–1643. [[CrossRef](#)] [[PubMed](#)]
24. Frankel, A.E.; Woo, J.-H.; Neville, D.M. Immunotoxins. In *Principles of Cancer Biotherapy*; Oldham, R.K., Dillman, R.O., Eds.; Springer: Dordrecht, The Netherlands, 2009; pp. 407–449.
25. Madhumathi, J.; Verma, R.S. Therapeutic targets and recent advances in protein immunotoxins. *Curr. Opin. Microbiol.* **2012**, *15*, 300–309. [[CrossRef](#)] [[PubMed](#)]
26. Ruiz-de-la-Herrán, J.; Tomé-Amat, J.; Lázaro-Gorines, R.; Gavilanes, J.G.; Lacadena, J. Inclusion of a Furin Cleavage Site Enhances Antitumor Efficacy against Colorectal Cancer Cells of Ribotoxin  $\alpha$ -Sarcin- or RNase T1-Based Immunotoxins. *Toxins* **2019**, *11*, 593. [[CrossRef](#)]
27. Tomé-Amat, J.; Ruiz-de-la-Herrán, J.; Martínez-del-Pozo, Á.; Gavilanes, J.G.; Lacadena, J.  $\alpha$ -sarcin and RNase T1 based immunoconjugates: The role of intracellular trafficking in cytotoxic efficiency. *FEBS J.* **2015**, *282*, 673–684. [[CrossRef](#)]
28. Van Daele, D.J.; Finnegan, E.M.; Rodnitzky, R.L.; Zhen, W.; McCulloch, T.M.; Hoffman, H.T. Head and neck muscle spasm after radiotherapy: Management with botulinum toxin A injection. *Arch. Otolaryngol. Head Neck Surg.* **2002**, *128*, 956–959. [[CrossRef](#)]
29. Layeeque, R.; Hochberg, J.; Siegel, E.; Kunkel, K.; Kepple, J.; Henry-Tillman, R.S.; Dunlap, M.; Seibert, J.; Klimberg, V.S. Botulinum toxin infiltration for pain control after mastectomy and expander reconstruction. *Ann. Surg.* **2004**, *240*, 608. [[CrossRef](#)]
30. Vasan, C.W.; Liu, W.-C.; Klusmann, J.-P.; Guntinas-Lichius, O. Botulinum toxin type A for the treatment of chronic neck pain after neck dissection. *Head Neck* **2004**, *26*, 39–45. [[CrossRef](#)]
31. Wittekindt, C.; Liu, W.-C.; Preuss, S.F.; Guntinas-Lichius, O. Botulinum toxin A for neuropathic pain after neck dissection: A dose-finding study. *Laryngoscope* **2006**, *116*, 1168–1171. [[CrossRef](#)]
32. Hartl, D.M.; Cohen, M.; Juliéron, M.; Marandas, P.; Janot, F.; Bourhis, J. Botulinum toxin for radiation-induced facial pain and trismus. *Otolaryngol. Head Neck Surg.* **2008**, *138*, 459–463. [[CrossRef](#)]

33. Stubblefield, M.D.; Levine, A.; Custodio, C.M.; Fitzpatrick, T. The role of botulinum toxin type A in the radiation fibrosis syndrome: A preliminary report. *Arch. Phys. Med. Rehabil.* **2008**, *89*, 417–421. [[CrossRef](#)] [[PubMed](#)]
34. Mittal, S.; Machado, D.G.; Jabbari, B. OnabotulinumtoxinA for treatment of focal cancer pain after surgery and/or radiation. *Pain Med.* **2012**, *13*, 1029–1033. [[CrossRef](#)]
35. Bach, C.A.; Wagner, I.; Lachiver, X.; Baujat, B.; Chabolle, F. Botulinum toxin in the treatment of post-radiosurgical neck contracture in head and neck cancer: A novel approach. *Eur. Ann. Otorhinolaryngol. Head Neck Dis.* **2012**, *129*, 6–10. [[CrossRef](#)]
36. Rostami, R.; Mittal, S.O.; Radmand, R.; Jabbari, B. Incobotulinum Toxin-A Improves Post-Surgical and Post-Radiation Pain in Cancer Patients. *Toxins* **2016**, *8*, 22. [[CrossRef](#)] [[PubMed](#)]
37. De Groef, A.; Devoogdt, N.; Van Kampen, M.; De Hertogh, L.; Vergote, M.; Geraerts, I.; Dams, L.; Van der Gucht, E.; Debeer, P. The effectiveness of Botulinum Toxin A for treatment of upper limb impairments and dysfunctions in breast cancer survivors: A randomised controlled trial. *Eur. J. Cancer Care* **2020**, *29*, e13175. [[CrossRef](#)] [[PubMed](#)]
38. Maily, M.; Benzakin, S.; Chauvin, A.; Brasnu, D.; Ayache, D. Radiation-induced head and neck pain: Management with botulinum toxin injections. *Cancer Radiother.* **2019**, *23*, 312–315. [[CrossRef](#)]
39. Oh, H.-M.; Chung, M.E. Botulinum toxin for neuropathic pain: A review of the literature. *Toxins* **2015**, *7*, 3127–3154. [[CrossRef](#)]
40. Park, J.; Park, H.J. Botulinum toxin for the treatment of neuropathic pain. *Toxins* **2017**, *9*, 260. [[CrossRef](#)]
41. Matak, I.; Bölskei, K.; Bach-Rojecky, L.; Helyes, Z. Mechanisms of botulinum toxin type A action on pain. *Toxins* **2019**, *11*, 459. [[CrossRef](#)]
42. Mittal, S.O.; Safarpour, D.; Jabbari, B. Botulinum toxin treatment of neuropathic pain. *Semin. Neurol.* **2016**, *36*, 73–83. [[CrossRef](#)]
43. Matak, I.; Lacković, Z. Botulinum neurotoxin type A: Actions beyond SNAP-25? *Toxicology* **2015**, *335*, 79–84. [[CrossRef](#)] [[PubMed](#)]
44. Vezdrevanis, K. Prostatic carcinoma shrunk after intraprostatic injection of botulinum toxin. *Urol. J.* **2011**, *8*, 239–241. [[PubMed](#)]
45. Ulloa, F.; González-Juncà, A.; Meffre, D.; Barrecheguren, P.J.; Martínez-Mármol, R.; Pazos, I.; Olivé, N.; Cotrufo, T.; Seoane, J.; Soriano, E. Blockade of the SNARE protein syntaxin 1 inhibits glioblastoma tumor growth. *PLoS ONE* **2015**, *10*, e0119707. [[CrossRef](#)] [[PubMed](#)]
46. He, D.; Manzoni, A.; Florentin, D.; Fisher, W.; Ding, Y.; Lee, M.; Ayala, G. Biologic effect of neurogenesis in pancreatic cancer. *Hum. Pathol.* **2016**, *52*, 182–189. [[CrossRef](#)] [[PubMed](#)]
47. Karsenty, G.; Rocha, J.; Chevalier, S.; Scarlata, E.; Andrieu, C.; Zouanat, F.Z.; Rocchi, P.; Giusiano, S.; Elzayat, E.A.; Corcos, J. Botulinum toxin type A inhibits the growth of LNCaP human prostate cancer cells in vitro and in vivo. *Prostate* **2009**, *69*, 1143–1150. [[CrossRef](#)] [[PubMed](#)]
48. Nam, H.J.; Kang, J.K.; Chang, J.S.; Lee, M.S.; Nam, S.T.; Jung, H.W.; Kim, S.-K.; Ha, E.-M.; Seok, H.; Son, S.W.; et al. Cells transformed by PLC-gamma 1 overexpression are highly sensitive to clostridium difficile toxin A-induced apoptosis and mitotic inhibition. *J. Microbiol. Biotechnol.* **2012**, *22*, 50–57. [[CrossRef](#)]
49. Proietti, S.; Nardicchi, V.; Porena, M.; Giannantoni, A. Botulinum toxin type-A toxin activity on prostate cancer cell lines. *Urologia* **2012**, *79*, 135–141. [[CrossRef](#)]
50. Bandala, C.; Perez-Santos, J.L.M.; Lara-Padilla, E.; Delgado Lopez, M.G.; Anaya-Ruiz, M. Effect of botulinum toxin A on proliferation and apoptosis in the T47D breast cancer cell line. *Asian Pac. J. Cancer Prev.* **2013**, *14*, 891–894. [[CrossRef](#)]
51. Bandala, C.; Cortés-Algara, A.L.; Mejía-Barradas, C.M.; Ilizaliturri-Flores, I.; Dominguez-Rubio, R.; Bazán-Méndez, C.I.; Floriano-Sánchez, E.; Luna-Arias, J.P.; Anaya-Ruiz, M.; Lara-Padilla, E. Botulinum neurotoxin type A inhibits synaptic vesicle 2 expression in breast cancer cell lines. *Int. J. Clin. Exp. Pathol.* **2015**, *8*, 8411–8418.
52. Rust, A.; Leese, C.; Binz, T.; Davletov, B. Botulinum neurotoxin type C protease induces apoptosis in differentiated human neuroblastoma cells. *Oncotarget* **2016**, *7*, 33220–33228. [[CrossRef](#)]

