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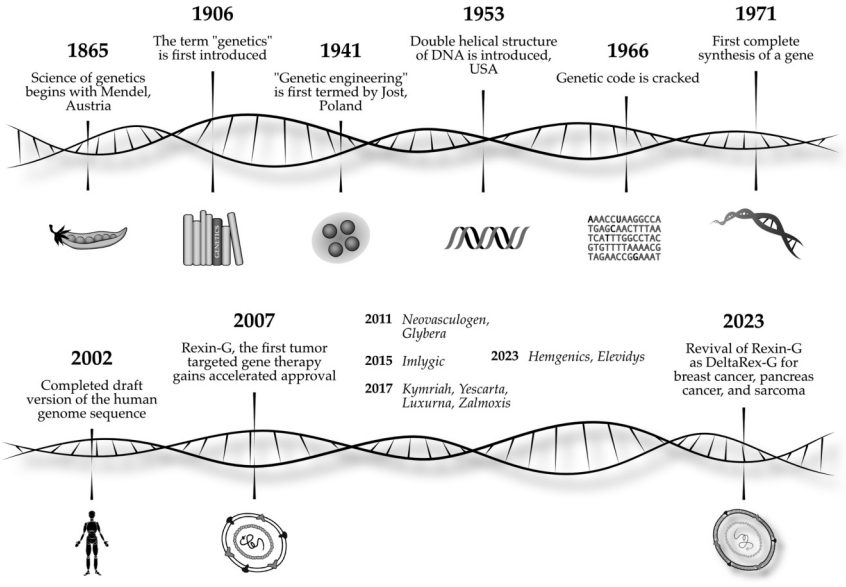
# From Mendel to Gene Therapy

SAMANTHA L. JEFFREY[1], DON A. BRIGHAM[2], SANT P. CHAWLA[3], NOAH FEDERMAN[1], FREDERICK L. HALL[2] and ERLINDA M. GORDON[2,3]

[1]*University of California, Los Angeles, Los Angeles, CA, U.S.A.;*
[2]*Aveni Foundation, Santa Monica, CA, U.S.A.;*
[3]*Cancer Center of Southern California/Sarcoma Oncology Research Center, Santa Monica, CA, U.S.A.*

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*Letter to the Editor*

# From Mendel to Gene Therapy

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[1]*University of California, Los Angeles, Los Angeles, CA, U.S.A.;*
[2]*Aveni Foundation, Santa Monica, CA, U.S.A.;*
[3]*Cancer Center of Southern California/Sarcoma Oncology Research Center, Santa Monica, CA, U.S.A.*

The 20th of July 2022 marked 200 years since the birth of Gregor Mendel, whose experimentation in the field of heredity laid the groundwork for modern genetics, consequently paving the way for gene therapy. Over the past bicentennial, the field of genetics has evolved from the study of inheritable traits in pea plants, to the implementation of revolutionary gene therapies with the aim of curing diseases that have plagued humanity since inception, with cancer and hemophilia at the forefront (Figure 1).

Gregor Mendel, an Austrian monk and teacher, spent nearly a decade studying patterns of inheritance (1). Ultimately settling on pea plants as his subject due to their distinguishable traits, ease of growing, and pace of reproduction, Mendel grew over 10,000 plants in the garden of his monastery over the course of his experiments (2). Implementing both cross-fertilization and self-fertilization methods, Mendel observed multiple generations of plant lineages, documenting seven distinct characteristics, and subsequently calculating the ratios of the two distinct forms of each characteristic. Analysis of this data led to the proposition of three principles of inheritance, which he presented in 1865 at the Natural History Society in Austria (3-5). Unbeknownst to Mendel, these three principles: the law of dominance, the law of segregation, and the law of independent assortment, accurately encompass the mode of inheritance of a multitude of human traits aptly referred to today as "Mendelian traits" (6, 7).

Although Mendel is now known as the "Father of Genetics", the term "genetics" was not introduced until 1906, decades after his studies (8, 9). William Bateson, who at the time served as Chair of Biology at Cambridge University, publicly established the term at the Third International Conference on Plant Hybridization during his inaugural address "The Progress of Genetic Research" (8, 10, 11). Three decades later in Poland, the term "genetic engineering" was coined by microbiologist A. Jost during a presentation in 1941 on yeast reproduction (12).

Links between DNA and the field of genetics date back to the early 1900s, yet DNA was not identified as the material of heredity until 1944 (13, 14). Within a decade, James Watson and Francis Crick published the basis of our modern understanding of DNA's structure: the double helix (15, 16). The article, published in 1953 in the journal Nature, detailed base pairing rules and the antiparallel nature of the sugar-phosphate backbones (15, 16). Watson and Crick's discovery ultimately arose from the x-ray crystallographic evidence collected by Rosalind Franklin, specifically an image taken by her student, PhD candidate Raymond Gossling, known as "Photo 51" (17, 18).

Francis Crick continued to make ground-breaking contributions to the field of genetics in the following years. In his laboratory, his team determined that three bases of DNA, now aptly referred to as a "codon", code for a singular amino acid (19, 20). By 1966, the contributions of multiple laboratories including Crick's, ultimately cracked the genetic code; the mRNA code for all 20 amino acids, in addition to both the start and stop codons, had been identified (20-23).

*Correspondence to:* Erlinda M. Gordon, Director, Gene and Cell Therapy/Immunotherapy, Sarcoma Oncology Research Center, LLC, Cancer Center of Southern California, 2811 Wilshire Blvd., Suite 414, Santa Monica, CA 90403, U.S.A. Tel: +1 3105529999 (Office), e-mail: egordon@sarcomaoncology.com

*Key Words:* Gregor Mendel, Watson & Crick, human genome project, DeltaRex-G, targeted gene delivery, Holy Grail of Gene Therapy, gene therapy, retroviral vector, adenoassociated vector, CAR-T cell therapy.

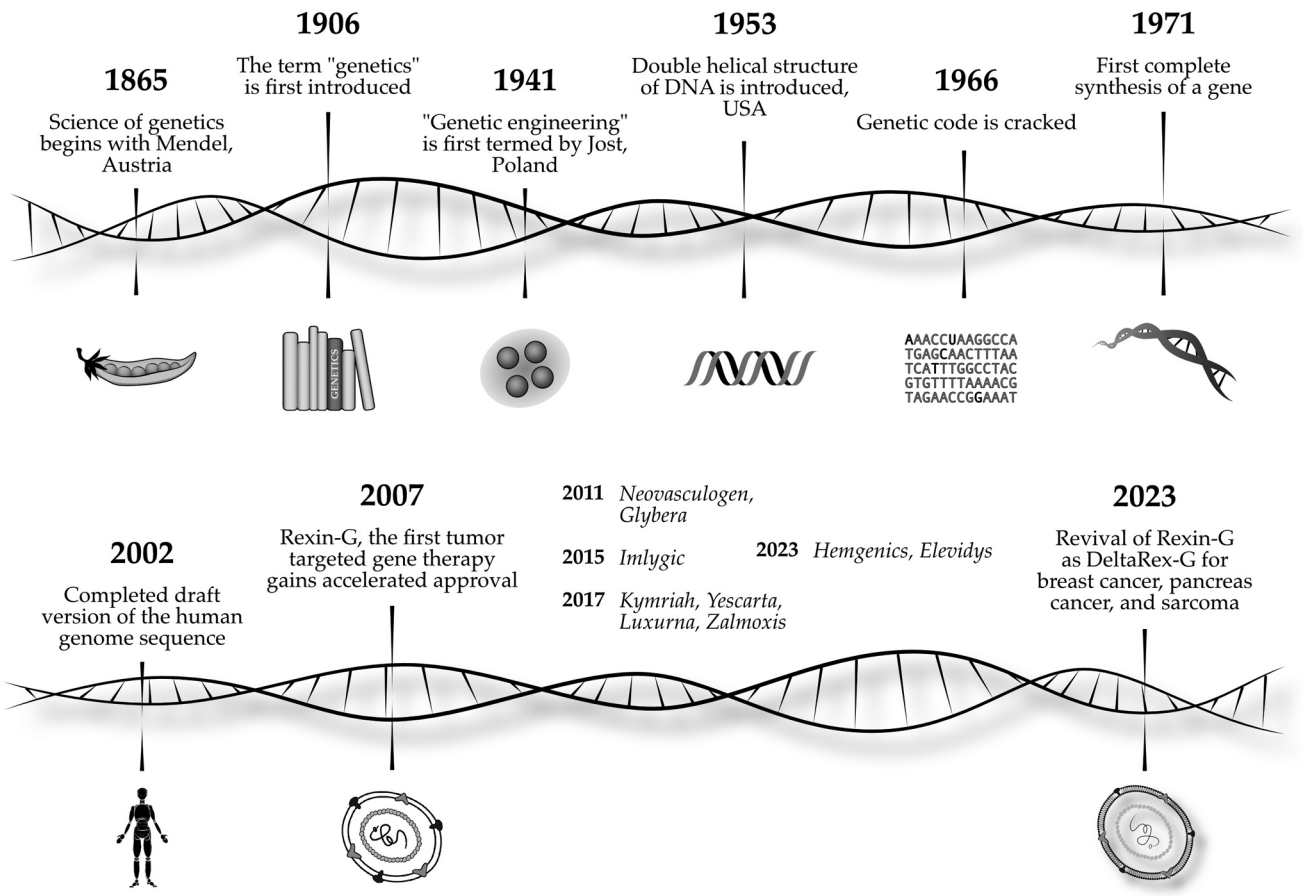


Figure 1. *Celebrating Mendel's 200th anniversary: Artist's illustration of the chronology of events from Gregor Mendel's experiments on pea plants to the discovery of the genetic code to genetic engineering of viral vectors for gene therapy applications (www.heathergordondrawings.com).*

Within a decade of the genetic code being solved, researchers at the University of Wisconsin reported accomplishing the first chemical synthesis of a gene (24, 25). Completed in 1970, the five year process involved the ligation of 17 segments of yeast DNA to form a 77-nucleotide-long gene encoding alanine tRNA (25, 26). The New York Times referred to this feat as a "new step along the road toward manipulation of the hereditary material in plants, animals, and perhaps, man" (25).

Arguably one of the largest accomplishments in modern science, The Human Genome Project released its final version of the first DNA sequence for (nearly) the entire human genome in 2003, after 13 years of international collaboration (27, 28). Though limited by the technology of the time, 90% of human DNA was successfully sequenced (27, 28). The database created by the Human Genome Project has allowed for the identification of over 2000 disease genes, including a multitude of genome sequences associated with cancer (29, 30).

In 2007, following decades of research in the fields of genetics and heredity, DeltaRex-G (Former name: Rexin-G, Mx-dnG1, dnG1), the first and so far the only tumor-targeted gene therapy received accelerated approval for all solid malignancies refractory to chemotherapy by the Philippine FDA following multiple demonstrations of safety and efficacy in advanced pancreatic cancer, cholangiocarcinoma, soft tissue sarcoma, osteosarcoma, breast cancer, colon cancer and prostate cancer (5, 31, 32). In 2009, the United States FDA (USFDA) granted DeltaRex-G fast-track designation for pancreatic cancer and orphan drug designation for soft tissue sarcoma and osteosarcoma (32).

DeltaRex-G, an intravenously injected retrovector, encodes a human cyclin G1 (CCNG1) inhibitor gene. CCNG1 is a non-canonical cyclin involved in tissue regeneration, but when pathologically activated as a proto-oncogene, CCNG1 activates the Mdm2 oncogene, which subsequently inactivates tumor suppressor p53, ultimately resulting in cancer progression (33, 34). Hence, inhibition of

CCNG1 indirectly restores the function of p53 by inactivating Mdm2 via the CCNG1 axis (34, 35). DeltaRex-G selectively binds to proteins and receptors in the tumor microenvironment (TME), and allows for the insertion of the CCNG1 inhibitor gene solely into tumor cells, leaving healthy cell populations untouched. The absence of collateral damage is achievable due to the inherent nature of retroviruses to only integrate into dividing cells, *i.e.*, cancer cells, proliferative neovasculature, and tumor-associated fibroblasts (TAFs) in the case of DeltaRex-G (5, 31, 36). After insertion, DNA is reverse-transcribed from the inserted RNA, and subsequently integrated into the cancerous cells' DNA, allowing for the expression of the CCNG1 inhibitor gene, consequently resulting in cell death via apoptosis-mediated pathways (5, 36). Further, due to enhanced CCNG1 expression in cancer stem cells and tumor-initiating cells that are capable of self-renewal, an interesting area of future research would investigate the role CCNG1 inhibition using DeltaRex-G may play in eradicating the cancer stem cells, which cause recurrence and metastasis (37). However, barriers to funding forced a stall in the continued development of DeltaRex-G for over a decade.

In the meantime, interest in gene therapy research and development significantly expanded, with the introduction of a variety of gene-therapy technologies to the international market. Notably, in 2015, talimogene laherparepvec (Imlygic), an oncolytic modified human Herpes simplex virus expressing a granulocyte macrophage colony stimulating factor (GM-CSF) transgene was approved by the USFDA for metastatic melanoma. Currently, talimogene laherparepvec has been shown to synergize with chemo-immunotherapy for advanced sarcoma (38). In 2017, a number of CAR-T cell products gained USFDA approval for hematologic malignancies, in which autologous T cells transfected with chimeric antigen receptors (CAR) *ex vivo* using retrovector technology (31, 39). Upon intravenous reintroduction, the genetically modified T cells selectively bind to proteins expressed by proliferating cancer cells, initiating apoptosis (31, 39). These CAR-T cell therapies [tisagenlecleucel (Kymriah) for acute lymphoblastic leukemia and axicabtagene ciloleucel (Yescarta) for large B-cell lymphoma] revolutionized the practice of cancer therapy, specifically immunotherapy, with demonstrations of treatment-induced remissions in patients with refractory leukemia and lymphoma (31). Recently, two adeno-associated viral vectors received USFDA approval: etranacogene dezaparvovec-drlb (Hemgenix), encoding the Factor IX gene, was approved for the treatment of hemophilia B, an X-linked bleeding disorder due to Factor IX coagulation factor deficiency and delandistrogene moxeparvovec-rokl (Elevidys), encoding a shortened functional segment of dystrophin, received accelerated approval for the treatment of Duchenne muscular dystrophy (38).

Finally, in July 2023, the USFDA Center of Biologics Evaluation and Research authorized the expansion of the Aveni Foundation Expanded Access program for advanced breast cancer in addition to pancreatic cancer and sarcoma, including the use of DeltaRex-G as a platform therapy upon which gene-targeted therapies and immunotherapies may be added. This authorization followed long-term survival studies demonstrating successfully prolonged lives of patients with advanced cancer, evidenced by >10- year survival of patients with pancreatic cancer, soft tissue sarcoma, osteosarcoma, breast cancer and B-cell lymphoma with DeltaRex-G therapy (25, 33, 40). Today, this targeted gene delivery system, long considered the "Holy Grail of Gene Therapy", has now become available again to cancer patients worldwide (5, 41-43).

Ultimately, the field of genetics has expanded exponentially since Mendel's pea plant experimentation in the mid-19$^{th}$-century, allowing for the discovery and development of life-saving gene therapy technologies. However, just as Mendel and his successors bore the pains of skepticism for years, the progression of gene therapy continues to face hindrance. Ethical concerns and regulatory measures pose barriers to the accessibility of these transformative technologies. Public acceptance, brought about by digestible research and education, as well as increased funding, are critical to expanding gene therapy research, opening exponential opportunities for further applications.

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b. Abstract not exceeding 250 words, organized according to the following headings: Background/Aim – Materials and Methods/Patients and Methods – Results – Conclusion. For Case Reports the structure should be as follows: Background/Aim – Case Report – Conclusion.

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e. Results (not needed in a Case Report);

f. Discussion;

g. Conclusion;

h. Conflicts of Interest;

i. Authors' Contributions;

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1 Kenyon J, Liu W, Dalgleish A: Report of objective clinical responses of cancer patients to pharmaceutical-grade synthetic cannabidiol. Anticancer Res 38(10): 5831-5835, 2018. DOI: 10.21873/anticanres.12924 (DOIs only if applicable)

2 McGuire WL and Chamnes GC: Studies on the oestrogen receptor in breast cancer. In: Receptors for Reproductive Hormones. O' Malley BW, Chamnes GC (eds.). New York City, NY, USA, Plenum Publ Corp., pp 113-136, 1973.

3 Global Health Estimates 2015: Disease Burden by Cause, Age, Sex, by Country and by Region, 2000-2015. Geneva, Switzerland, World Health Organisation, 2016. Available at: **http://www.who.int/healthinfo/global_burden_disease/estimates/en/index2.html** [Last accessed on April 3, 2018] (The web address should link directly to the cited information and not to a generic webpage)


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