

Chapter 1

Targeted Drug Delivery in General, New Technology in Medicine

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1.1 Introduction

The term “silver bullet” originates from folklore in which a silver bullet was the only type of bullet for firearms effective against a number of mystical foes. In modern vocabulary its use refers to a simple fix for a complex or intractable problem. In search of such solutions, the attention of investigators to pharmacologics which specifically target disease while leaving the host otherwise untouched has evolved.

A medline search of “targeted therapy” or “targeted drug delivery” reveals two articles from 1902–1978 with the first mention attributed to the use of propranolol for essential hypertension by Lauro *et al.*¹ In the 1980’s 53 articles were published with the concept of targeted approaches which blossomed in the 1990’s to 261. Since that time significant expansion in this field has occurred with 1429 articles for targeted approaches from 2000 to 2006. While these references refer to a wide variety of diseases the preponderance deals with potential solutions for the treatment of cancers. This explosion of information in targeting is likely attributable to our increasing knowledge of disease, in particular cellular mechanisms and receptors, as well as the technological advances in vehicles for drug delivery.

The purpose of this chapter is to provide the reader with a historical context of the fields of research which have contributed to this revolution while leaving the in depth discussion of devices, methodologies, and current approaches to other chapters which specifically deal with these subjects.

1.2 Historical Perspective

The current state of targeted therapy technology takes root in collaborative efforts from a number of physical, biological and medical disciplines under the broad umbrella of nanotechnology. The birth of nanotechnology is usually attributed to a talk given by physicist Richard Feynman in 1959, “There’s Plenty of Room at the Bottom” at the American Physical Society. He suggested concepts such as small robots building smaller robots resulting in the creation of billions of tiny factories and a reduction in print type size to permit the Encyclopedia Britannica to be fit to the size of a pin head. The term nanotechnology was actually coined in 1974 by Professor Noro Taniguchi of the Tokyo Science University. By strict definition, nanotechnology refers to molecular devices smaller than 1 micron and therefore on the “nano” scale. One nanometer is one billionth or 10^{-9} of a meter.

Soon after mechanical and electrical approaches became feasible, biologists began to explore opportunities for advancement. In 1996 the first scientific conference entitled, “Biological Approaches and Novel Applications for Molecular Nanotechnology” was held. The resulting concept of nanomedicine has emerged as an offshoot of nanotechnology referring to highly specific medical intervention at the molecular scale for curing disease or repairing damaged tissues, such as bone, muscle, or nerve.² It combines the expertise of individuals in medicine, biology, math, chemistry, engineering, and computer science for the creation of devices to meet these needs. Institutes for nanomedicine sponsored by the national institutes of health, universities, and the private sector have arisen as an outcome of this direction.

1.3 Targeted Drug Delivery

While all advances associated with targeted drug therapy are not strictly bound to nanomedicine, most of the current progress is within this realm and is the focus of this text. It should be recognized that targeted therapies are different from “passive targeting”. Examples of passive targeting included technologies such as “enhanced permeability and retention (EPR)” and the products created by the Alza Corporation which seeks to overcome the body’s natural barriers to drug entry and extend the time in which drugs remain at their site of action. Nanomedicine targeted approaches attempt to interact with the cell in a number of complex ways to offer advantage over untargeted approaches which treat all cells the same. It is important to recognize that for targeting to occur, some selective process must take place whereby normal host tissues are not affected in similar ways to the tissues in which the desired effect is to take place. While this introduction can in no form be exhaustive, some global approaches to this problem have included:

1. Systemic delivery of agents with a selective advantage to cells which abnormally express or overexpress a surface or intracellular receptor that is targeted.
2. Delivery of agents which replace disease rendering defective biochemical processes with competent alternatives (gene therapy).
3. Delivery of agents or devices which inhibit specific cellular pathways in targeted cells.
4. Immune boosting for direct destruction of desired cells (immunotherapy).

Given the confines of this chapter as an overview and the fact that the majority of targeted approaches are directed toward tumor biology, the examples given will come from this realm. It is important for the reader to realize that these concepts have been globalized to many other disease processes. Also of importance is that few examples will be offered in each realm which is representative of a large body of pre-clinical and clinical evidence for which volumes could be dedicated to each individual subject.

1.4 Systemic Delivery of Agents with a Selective Advantage to Cells which Abnormally Express or Overexpress a Surface Receptor that is Targeted

Advances in cancer research have resulted in significant understanding of the cellular and molecular changes leading to malignancy. The concepts of tumor progression were elaborated by Foulds in the 1950's³ which was shortly followed by evidence from cytogenetics in the 1960's. Molecular techniques evolved and revealed that tumorigenesis results from a single altered cell.⁴ Ultimately a multi-step model with acquisition of various cellular abnormalities was proposed.⁵ While all of the steps involved at present remain unknown, our understanding of the cancer cell and of the role of the surrounding network of tissues is increasing.⁶ Markers of pre-malignancy and malignancy have been identified in some cases and targeted approaches are being made to utilize these markers in treatment strategies. In addition, other nontransforming markers which are overexpressed for a variety of reasons may be targeted to offer some selective advantages in treatment.

A preclinical example of this process has evolved through the creation of targeted dendrimer therapy. Dendritic macromolecules, or dendrimers, are uniformed spherical nano-structures ranging from 10 to 200 Angstroms in diameter. Dendrimers have been used as a backbone for the attachment of several types of biological materials including folate. These molecules can direct the dendrimers to locations where receptors are expressed on tumor cell surfaces. Dendrimers can also be conjugated to therapeutic molecules, such as methotrexate, allowing for efficacious delivery and a higher dose load to tumor cells avoiding the systemic toxicity of current therapeutic strategies.⁷

Dendrimers with folate and methotrexate have been shown to bind to KB cells expressing high levels of folate binding protein. In animal model studies targeted chemotherapy with dendrimers showed ten times the efficacy and decreased toxicity compared to standard chemotherapy with free drug.⁸ Phase I clinical trials for this therapy are planned.

Using this technology one can envision a multitude of devices whereby both the targeted agent and the chemotherapeutic agent utilized could be specifically modified to meet the need of the receptor characteristics and physiological properties of an individual tumor. In support of this concept, additional dendrimers which target epidermal growth factor receptor (EGFR), prostate specific membrane antigen (PSMA), and RGD peptides have been fabricated and tested as well as conjugates with Taxol as the therapeutic arm. In this scenario, patient tumors could be screened for their cell surface receptor levels in order to make decisions regarding which therapeutic would have greatest potential.

Another targeting approach for cellular receptors comes from the work with monoclonal antibodies for targeting of receptors. EGFR which is presently being targeted in colorectal cancer as well as head and neck cancer (HNSCC) with a number of other tumors currently under study. EGFR is a very desirable target in HNSCC with mRNA overexpression present in approximately 92% of specimens studied.⁹ EGFR overexpression in HNSCC is the result of both decreased receptor down regulation and increased mRNA synthesis.¹⁰ Following ligand binding, EGFR is internalized and found in the intracellular compartment and ultimately the nucleus. Targeted approaches for EGFR have been studied in clinical trails where antibodies directed to EGFR have shown promise as an adjunct to radiation therapy. Bonner *et al.* studied in a randomized prospective multinational trial the effect of cetuximab (monoclonal antibody to EGFR) and radiation vs. radiation alone. With cetuximab, median survival, progression free survival and local regional control of disease were all increased.¹¹ Based in large part on these results, the FDA in 2006 approved cetuximab as the first new treatment in 45 years for head and neck cancer.

1.5 Delivery of Agents which Replace Disease Rendering Defective Biochemical Processes with Competent Alternatives (Gene Therapy)

Our understanding of cellular processes associated with disease has made possible the creation of therapeutics which has potential to enter all cells but replace missing components in defective cells such that they only exhibit their effect in this environment. An example of such a strategy

includes the use of gene therapy in which viral and nonviral vectors transfer genetic material to diseased cells.

Attempts at gene therapy have resulted in some success and many challenges in need of further exploration to create an effective treatment. Despite its current clinical limitations, a number of solid tumors including pancreatic, breast, colon, lung, prostate and head and neck cancer have been successfully treated in mouse models.¹²⁻¹⁴

In clinical practice a recombinant adenovirus has been used to deliver competent p53 (TP53 gene) to cells which are p53 deficient. p53 is a tumor suppressor gene which monitors DNA damage, inducing cell cycle arrest for repair or apoptosis when necessary.¹⁵ p53 inactivation has been demonstrated in up to 50% of all human cancers.

Gene therapy has been attempted for cells with mutated p53 with the use of an adenoviral vector containing wild-type p53.¹⁶ In clinical study, 33 patients had injection of Adenovirus-p53 (Ad-p53) intratumorally without any evidence of toxicity. Seventeen non resectable patients could be evaluated for clinical efficacy. Two patients demonstrated greater than 50% response and an additional six had stable disease for up to 3.5 months. Nine patients had unabated progression of disease. An additional six patients showed stable disease for up to 3.5 months. Nine patients had unabated progression of disease.

Another growing area related to viral gene therapy includes the use of oncolytic vectors for cancer cell destruction while leaving the normal cells in the body unaffected. A number of different viruses have been studied for this purpose including vaccinia, adenovirus and herpes simplex

virus. A number of animal trials using this technology have shown promising results.^{17,18}

HSV-1 has been used with the production of two vectors G207 and NV1020 in phase I and II clinical trials after success *in vivo* and animal models.^{19,20} ONYX-015, an adenovirus which can only replicate in cells lacking functional p53. Khuri and Nemunaitis *et al.*²¹ reported on a non-randomized phase II trial of 37 patients with multiple recurrent tumors who received ONYX-015. Thirty patients could be evaluated for disease response. Treatment with ONYX-015 caused tumors to shrink in 25 of the 30 cases with greater than 50% response in 63% of the patients. There were 8 complete and 11 partial responses and in some patients with tumors as large as 10 cm in diameter complete regression was noted. At six months none of the responding tumors had progressed.

1.6 Delivery of Agents or Devices which Inhibit Specific Cellular Pathways Present in Targeted Cells

Molecular targeted therapeutics in this category includes nucleic acid drugs (antisense oligonucleotides and small inhibitory RNA) and small molecule inhibitors. Antisense oligonucleotides are single stranded DNA or RNA of approximately 20 nucleotides in length. RNA antisense oligonucleotides are EXON regions of the desired mRNA to block ribosomal translation and therefore protein production.²² DNA antisense oligonucleotides bind to complementary RNA creating a DNA/RNA hybrid which is subsequently degraded. Both pathways have the potential for gene silencing.

Small interfering RNA's (siRNA's) are double stranded RNA molecules which also have the potential to block protein production. They can be directly introduced into cells via virus or experimental manipulation. Double stranded RNA in the cell is cleaved by the Dicer enzyme into 21–28 nucleotide sequences which form RNA-induced silencing complexes through their association with DSRNA-binding protein R2D2.²³

In pre-clinical models siRNA targets have been tested in a number of cancers including breast, liver, esophagus, and melanoma.²⁴ As a recent technology, reports of human clinical trials for this therapy have not yet emerged.

Another example of pathway targeting comes from the Philadelphia Chromosome in chronic myelogenous leukemia which was discovered in the 1960's. This chromosomal translocation between chromosomes 9 and 22 leads to a fusion protein bcr-abl which becomes a continuously active tyrosine kinase associated with the disease. Imatinib (Gleevec[®], Novartis) takes its mechanism of action by binding to bcr-abl at the ATP binding site thereby inhibiting the activity of the protein. While the abl tyrosine kinase is also inhibited in other cells its effect is more profound in CML due to its dependence on high levels of the abnormal protein. Although eradication of CML is not achievable, Imatinib limits the growth of the tumorigenic cells and decreases the risk of blastic crisis.

1.7 Immune Boosting for Direct Destruction of Desired Cells

Immunotherapy in general involves approaches to expand and activate the immune system to target cancer cells for tumor control. To date,

CD8+ cytolytic T lymphocytes (CTL) appear to play the key role in tumor response which is supported by the CD4+ T helper cells. Both cells receive information from antigen presenting cells for activation against potential immunogenic peptides for tumor control. Augmenting this system through exogenous antigen presentation and the boosting of cellular signals for increased activation has been studied extensively and continues to show promise in clinical application.

Current body sites for clinical trials for cancer immunotherapy include prostate, pancreas, melanoma, and kidney. In melanoma, MDX-010 anti-CTLA-4 and IL-2 are used to augment and prolong T-cell antitumor response in preclinical and clinical phase I/II studies.²⁵ An additional approach has been combination therapy with anti-CTLA-4 and vaccine therapy.²⁶

Treatment for renal cell cancer highlights a number of approaches. Greater than 20 years experience with high dose Interleukin-2 (IL-2) as an immunomodulator has been conclusively shown to give complete regression in 5–7% of candidates for therapy.²⁷ Additional areas of research in this arena include T-Cell transfer whereby T-cells for *ex vivo* expansion are harvested from and later delivered to the patient. Combination of this approach with cloning of cells which recognize p53, NY-ESO-1, and other antigens has shown promise.²⁸

1.8 Conclusion and Future Direction in Research and Technology

Advances in targeted drug delivery are likely to continue to change treatment approaches in the near- and long-term future. As our understanding

of the complex processes involved in the diseased cell increases, new opportunities for targeting will emerge. Targets at present come from a variety of sources including both normal and abnormal cellular receptors and biochemical processes. When normal cellular processes are involved, selective advantage is offered by an upregulation of these processes in the tumor cell compared to normal tissue. This allows tumor cells to be more highly affected than their normal counterparts. Abnormal receptors and processes also offer an opportunity for targeting in that they do not have counterparts within normal tissue.

Development of new and the expansion of currently available strategies hold great promise in cancer and all diseases. Approaches for targeting will continue to expand both as our knowledge of potential targets increases and as our ability to create targeted therapeutics continues to expand. With accelerating speed it is likely that the trend towards increasingly specific treatment will remain a desirable outcome long into the future of nanomedicine. The limited number of strategies reviewed here has provided only a small number of examples of an ever growing field of science in targeted therapeutics for drug delivery.

1.9 References

1. R. Lauro, A. Platania, C. Liberatore, G. Reda, and C. Spinelli, Biochemical profile of essential arterial hypertension. Indications for a targeted therapy: Experience with propranolol, *Clinica Terapeutica*, **85**(1), 19–25, 1978.
2. NIH Roadmap for Medical research, <http://nihroadmap.nih.gov/nanomedicine/>

3. L. Foulds, Tumor progression, *Cancer Research*, **17**, 355–356, 1957.
4. P. C. Nowell, The clonal evolution of tumor cell populations, *Science*, **194**, 23–28, 1976.
5. B. Vogelstein and K. W. Kinzler, The multistep nature of cancer, *Trends Genet.*, **9**, 138–141, 1993.
6. D. Hanahan and R. A. Weinberg, The hallmarks of cancer, *Cell*, **100**, 57–70, 2000.
7. A. Quintana, E. Raczka, L. Piehler, I. Lee, A. Myc, I. Majoros, A. Patri, T. Thomas, J. Mulé, and J. R. Baker Jr., Design and function of a dendrimer-based therapeutic nanodevice targeted to tumor cells through the folat receptor, *Pharmaceutical Research*, **19**, 1310–1316, 2002.
8. J. Kukowska-Latallo, K. A. Candido, Z. Cao, S. S. Nigavekar, I. J. Majoros, T. P. Thomas, L. P. Balogh, M. K. Khan, and J. R. Baker Jr., Nanoparticle targeting of anticancer drug improves therapeutic response in animal model of human epithelial cancer, *Cancer Research*, **65**, 5317–5324, 2005.
9. J. R. Grandis and D. J. Tweardy, Elevated level of transforming growth factor alpha and epidermal growth factor receptor messenger RNA are early markers of carcinogenesis in head and neck cancer, *Cancer Research*, **53**, 3579–3584, 1993.
10. J. R. Grandis, Q. Zeng, S. D. Drenning, and D. J. Tweardy, Normalization of EGFR mRNA levels following restoration of wild-type p53 in a head and neck squamous cell carcinoma cell line, *Int. J. Oncol.*, **13**, 375–378, 1998.
11. J. A. Bonner, P. M. Harari, J. Giralt, N. Azarnia, D. M. Shin, R. B. Cohen, C. U. Jones, R. Sur, D. Raben, J. Jassem, R. Ove, M. S. Kies,

- J. Baselga, H. Youssoufian, N. Amellal, E. K. Rowinsky, and K. K. Ang, Radiotherapy plus cetuximab for squamous-cell carcinoma of the head and neck, *N. Engl. J. Med.*, **354**, 567–578, 2006.
12. J. F. Tseng and R. C. Mulligan, Gene therapy for pancreatic cancer, *Surg. Oncol. Clin. N. Am.*, **11**, 537–569, 2002.
 13. J. W. Rocco, D. Li, W. H. Liggett Jr., L. Duan, J. K. Saunders Jr., D. Sidransky, and B. W. O'Malley Jr., p16INK4A adenovirus-mediated gene therapy for human head and neck squamous cell cancer, *Clin. Cancer Res*, **4**, 1697–1704, 2004.
 14. S. Varghese and S. D. Rabkin, Oncolytic herpes simplex virus vectors for cancer virotherapy, *Cancer Gene Ther.*, **9**, 967–978, 2002.
 15. B. J. Baum, M. Kok, S. Tran, and S. Yamano, The impact of gene therapy on dentistry a revisiting after six years, *JADA*, **133**, 35–44. 2002.
 16. G. L. Clayman, A. K. el-Naggar, S. M. Lippman, Y. C. Henderson, M. Frederick, J. A. Merritt, L. A. Zumstein, T. M. Timmons, T. J. Liu, L. Ginsberg, J. A. Roth, W. K. Hong, P. Brusio, and H. Goepfert, Adenovirus-mediated p53 gene transfer in patients with advanced recurrent head and neck squamous cell carcinoma, *J. Clin. Oncol.*, **16**, 2221–2232, 1998.
 17. A. Hemminki, A. Kanerva, E. J. Kremer, G. J. Bauerschmitz, B. F. Smith, B. Liu, M. Wang, R. A. Desmond, A. Keriell, B. Barnett, H. J. Baker, G. P. Siegal, and D. T. Curiel, A canine conditionally replicating adenovirus for evaluating oncolytic virotherapy in a syngeneic animal model, *Mol. Ther.*, **7**, 163–173, 2003.
 18. P. J. Cozzi, S. Malhotra, P. McAuliffe, D. A. Kooby, H. J. Federoff, B. Huryk, P. Johnson, P. T. Scardino, W. D. Heston, and Y. Fong,

- Intravesical oncolytic viral therapy using attenuated, replication-competent herpes simplex viruses G207 and Nv1020 is effective in the treatment of bladder cancer in an orthotopic syngeneic Model, *FASEB J.*, **15**, 1306–1308, 2001.
19. R. Liu, S. Varghese, and S. D. Rabkin, Oncolytic herpes simplex virus vector therapy of breast cancer in C3(1)/SV40 T-antigen transgenic mice, *Cancer Research*, **65**, 1532–1540, 2005.
 20. J. J. Bennett, K. A. Delman, B. M. Burt, A. Mariotti, S. Malhotra, J. Zager, H. Petrowsky, S. Mastorides, H. Federoff, and Y. Fong, Comparison of safety, delivery, and efficacy of two oncolytic herpes viruses (G207 and NV1020) for peritoneal cancer, *Cancer Gene Ther.*, **9**, 935–945, 2002.
 21. F. R. Khuri, J. Nemunaitis, I. Ganly, J. Arseneau, I. F. Tannock, L. Romel, M. Gore, J. Ironside, R. H. MacDougall, C. Heise, B. Randlev, A. M. Gillenwater, P. Brusco, S. B. Kaye, W. K. Hong, and D. H. Kim, A controlled trial of intratumoral ONYX-015, a selectively replicating adenovirus, in combination with cisplatin and 5-fluorouracil in patients with recurrent head and neck cancer, *Nat. Med.*, **6**(8), 879–885, 2000.
 22. H. Wang, G. Prasad, J. K. Buolamwini, and R. Zhang, Antisense anticancer oligonucleotide therapeutics, *Curr. Cancer Drug Targets*, **1**, 177–196, 2001.
 23. Y. Dorsett and T. Tuschl, siRNAs: Applications in function genomics and potential as therapeutics, *Nat. Rev. Drug Discovery*, **3**, 318–329, 2004.
 24. M. Izquierdo, Short interfering RNAs as a tool for cancer gene therapy, *Cancer Gene Ther.*, **12**, 217–227, 2004.

25. A. V. Maker, G. Q. Phan, P. Attia, and J. C. Yang *et al.*, Tumor regression and autoimmunity in patients treated with cytotoxic T-lymphocyte-associated antigen 4 blockade and interleukin 2: A phase I-II study, *Ann. Surg. Oncol.* **12**(12), 1005–1016, 2005.
26. A. van Elsas, R. P. Suttmuller, A. A. Hurwitz, J. Ziskin, J. Villasenor, J. P. Medema, W. W. Overwijk, N. P. Restifo, C. J. Melief, R. Offringa, and J. P. Allison, Elucidating the autoimmune and anti-tumor effector mechanisms of a treatment based on cytotoxic T lymphocyte antigen-4 blockade in combination with a B16 melanoma vaccine: Comparison of prophylaxis and therapy, *J. Exp. Med.*, **194**, 481–489, 2001.
27. S. A. Rosenberg, J. C. Yang, D. E. White, and S. M. Steinberg, Durability of complete responses in patients with metastatic cancer treated with high-dose interleukin-2: Identification of antigens mediating response, *Ann. Surg.*, **228**, 307–319, 1998.
28. C. J. Cohen, Z. Zheng, R. Bray, Y. Zhao, L. A. Sherman, S. A. Rosenberg and R. A. Morgan, Recognition of fresh human tumor by human peripheral blood lymphocytes transduced with a bicistronic retroviral vector encoding a murine anti-p53 TCR. *J. Immunol.*, **175**, 5799–5808, 2005.