Expert Opinion

Digestive enzyme targeted polymer therapeutic: MIT WO2007103364

Richard A Gemeinhart
University of Illinois, College of Pharmacy, Department of Biopharmaceutical Sciences, 833 South Wood Street (MC 865), Chicago, IL 60612-7231, USA

The basic claims of this application involve methods that use a polymer to deliver therapeutic molecules in a targeted fashion using a polymeric carrier, drug and a biologically degradable linker. The release of therapeutic molecule is in response to digestive enzymes that are overexpressed in diseased tissues in the body. Further claims, of the sixteen included, define the digestive enzymes and drug–polymer linkages that may be used and specify tumors as the disease of particular interest. Preclinical examples presented support the idea that this therapeutic design has merit. There does not seem to have a single clinical candidate but future work is expected to develop these molecules into clinical products for cancer and particularly cancer treatment combined with radiation.

Keywords: cancer, polymer, polymer therapeutic, protease

Expert Opin. Ther. Patents (2008) ***18****(9):1085-1090*

1. Introduction

In drug discovery and pharmacology, a drug target can be described as a "molecular structure (chemically definable by at least a molecular mass) that will undergo a specific interaction with chemicals that we call drugs because they are administered to treat or diagnose a disease" [1]. From a drug design standpoint, this definition of a drug target or molecular target has been used to identify new chemical entities (NCEs) that directly treat the disease. Once that NCE has been verified to be a drug candidate, the NCE is then considered an active pharmaceutical ingredient (API) in a human formulation, which is used to deliver the drug. However, from the drug delivery standpoint, drug targeting can be concisely defined as any method that optimizes the therapeutic index of an API by using a drug carrier that recognizes attributes of the target tissue.

More specifically, targeted drug delivery involves increasing the local concentration of the API in the location of the disease although minimizing the API concentration in locations of toxicity [2,3]. In no disease is targeted drug delivery system development progressing at a greater rate than cancer [4] and this is the example disease that is described in the example patent examined; however, the patent is careful to claim no specific disease. The focus is primarily owing to the deadly nature of cancer. But not only is the disease deadly but many of the most active APIs for treating cancer are not easily delivered owing to chemical and physical characteristics of the APIs and the APIs have severe systemic toxicity. The vast majority of existing anticancer APIs act by killing cells, or causing apoptosis in cells that are most rapidly dividing [5] and thus need strategies developed to minimize the toxicity; that is, cancer APIs need targeted drug delivery!

By examining the biologic and physical characteristics of the tumor, Ringsdorf most famously proposed a model for drug targeting in the mid-1970s [6] that is still being developed (**Figure 1**). In this scheme, an API is bound to a polymer

informa
healthcare

10.1517/13543770802358180 © 2008 Informa UK Ltd ISSN 1354-3776

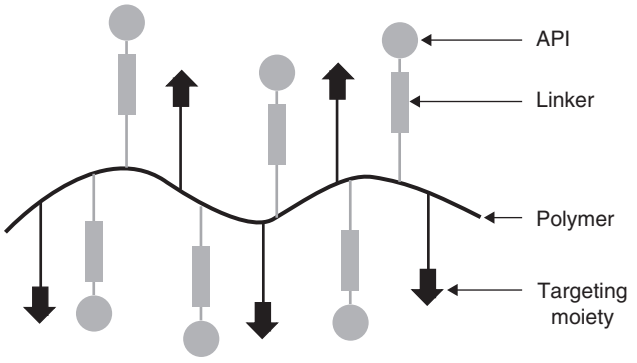
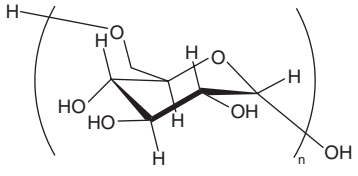
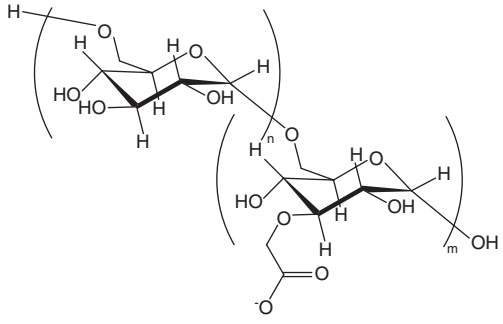


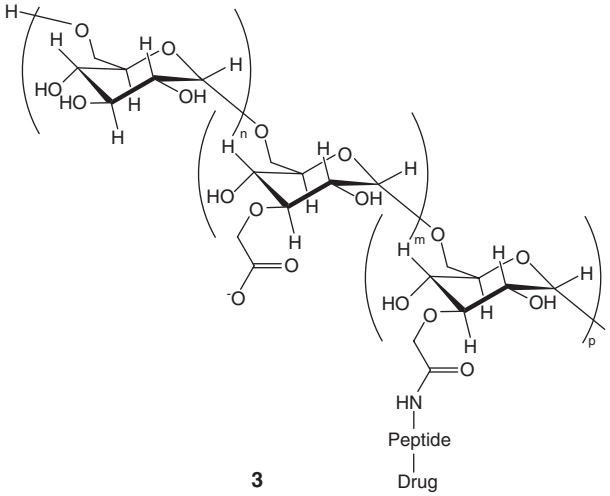
Figure 1. Schematic representation of the Ringsdorf model of polymer therapeutics. APIs, or drugs, and targeting moieties are attached to a polymer backbone. A single or several APIs and targeting moiety can be present on the polymer chain.
API: Active pharmaceutical ingredient.



1



2



3

through a cell-activated bond creating a new chemical entity that is polymeric in nature. In addition to the API being attached to the polymer, other molecules could be included that would allow the polymer to be selectively internalized by a specific cell type or localized to a specific cell type. This type of targeting in which a biochemical binding to the diseased cells is present is referred to by most as active targeting. Polymeric drug delivery systems that utilize the various components of Ringsdorf model – polymer–API linker and targeting entity – together or separately can be classified as polymer therapeutics [7].

In addition to active targeting, polymer therapeutics and nanometer scale particles – on the order of 200 nm – accumulate in tumors owing to the open vascular epithelium and reduced lymphatic drainage commonly referred to as the enhanced permeation and retention effect [8,9] although this effect is not uniform throughout tumor types [10]. This physical accumulation of the targeted system in the diseased tissue is referred to as passive targeting. Therefore, polymer therapeutics can have up to four levels of drug targeting: i) if a targeting moiety is conjugated to the polymer; ii) if the linker is selective for the diseased cells; iii) if the polymer therapeutic is appropriate for the enhanced permeation and retention effect; and iv) if the API has an appropriate molecular target. But these four targeting levels are not the only advantages of polymer therapeutics; polymeric system have extended blood retention owing to the macromolecular nature of the system being greater than the glomerular filtration of the kidney [11,12]. Therefore, polymeric systems are now widely being pursued for therapeutic applications.

The patent application that forms the basis of this evaluation is from the Massachusetts Institute of Technology and claims any and all polymer carriers with a drug attached through a linker that is cleaved by a digestive enzyme. The original Ringsdorf model and most of the research so far focused on intracellular linker cleavage and drug activation [4,7,13] whereas this patent focuses on extracellular degrading enzymes. The general idea that is protected is that of the Ringsdorf model but the linker is specified as one that is cleaved by digestive enzymes and the targeting moiety is omitted, or more appropriately described; it is replaced with a linker that is the targeting moiety. Therefore, there are only three of the four levels of targeting in this system.

2. Chemistry

Within this patent, the specific chemistry used was not a claim nor are any of the chemistries novel chemistries. Several examples are given in which chemistries are described that are shown to be important for the activity of the claimed conjugates. The chemistry used to modify the dextran (**1**) to create carboxymethyl dextran (**2**), drug-conjugated carboxymethyl dextran (**3**), ethanolamine-modified carboxymethyl dextran and drug-conjugated ethanolamine-modified carboxymethyl dextran has been previously reported [14]. As

Table 1. Chemical and enzymatic properties of the dextran polymer, carboxymethyl dextran conjugates, peptide substrate and peptide prodrugs.

Polymer	Peptide	ζ-Potential (mV)‡	k_{cat}/K_m (M^{-1} s^{-1})*
Dextran	–	-6.6	–
C1	PVGLIG-MTX	-27.8	1.09×10^4
C2	PVGLIG-MTX	-12.2	1.22×10^5
–	IPVSLRSG	–	8.2×10^4
–	IPVGLIG-Dox	–	1.4×10^5

*Kinetic parameters for MMP-2 summarized from Tables 2 and 6 of the patent. No cleavage kinetics can be calculated if no peptide is present.
‡ζ-Potential summarized from Tables 5 and 6 of the patent. No ζ-potential can be calculated for the peptide or peptide–drug conjugate.
C1: Ethanolamine-modified, carboxymethyl dextran peptide–drug conjugate with low ethanolamine substitution; C2: Ethanolamine-modified, carboxymethyl dextran peptide–drug conjugate with higher ethanolamine substitution; Dox: Doxorubicin; k_{cat}: Catalytic constant; K_m: Michaelis constant; MTX: Methotrexate.

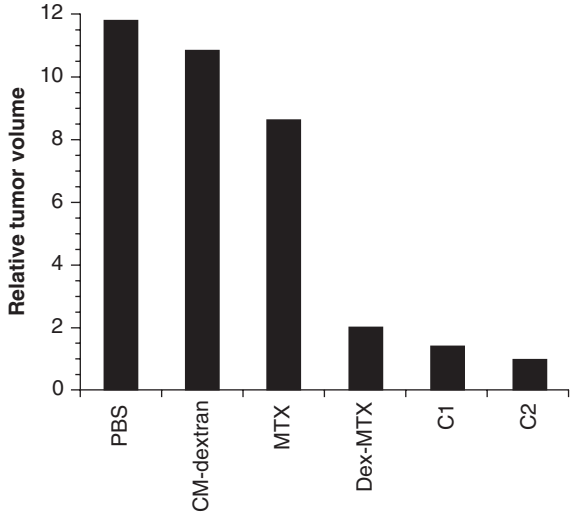


Figure 2. Relative tumor size as determined by the ratio of tumor size on day 18 or 19 and the initial tumor size on day 1. The data were extracted from Figures 13 and 15 from the patent. The number of animals was reported to be three and no error bars were apparent. C1 and C2 correspond to the ethanolamine-modified carboxymethyl dextran conjugates described in **Table 1**.
C1: Ethanolamine-modified, carboxymethyl dextran peptide–drug conjugate with low ethanolamine substitution; C2: Ethanolamine-modified, carboxymethyl dextran peptide–drug conjugate with higher ethanolamine substitution; CM-dextran: Carboxymethyl dextran; Dex-MTX: Dextran–methotrexate; MTX: Methotrexate.

previously reported, the carboxymethylation of dextran was ~ 50% in all reports and the ethanolamine modification was stoichiometric. Published results for a methotrexate–carboxymethyl dextran were summarized. A new conjugate, doxorubicin–carboxymethyl dextran, was reported using methods similar in design to the previously reported method for methotrexate.

3. Biology and action

The biologic activity of the dextran–methotrexate conjugates have been reported previously [14-17] but will be summarized here. Of primary importance in the examples is the fact that charge neutralization had a significant impact on the cleavage of the peptide-based conjugates (**Table 1**). The observation was that a more neutral polymer carrier, compared with the electronegative carboxymethyl dextran, allowed a more specific cleavage, that is, higher k_{cat}/K_m. The patent clearly indicates that "none of the peptide motifs of the MMP-2 recognition sequence found in the literature carry a negatively charged amino acid" [18,19]. Several patents and published substrates used for drug or imaging activation agree with this observation whether they are based on the published substrates or used phage display techniques to identify novel substrates [20-25]. This is owing to the hydrophobic pocket and recognition sequence of MMP-2 with predominantly hydrophobic residues in the binding pocket (Ala, Leu, Gly, Ile) and the zinc binding domain [26].

Beyond cleavage, the peptide–doxorubicin and peptide–methotrexate conjugates were confirmed to be active toward an array of cell lines with similar activity as the free drug. In addition to showing that peptidyl–drug and the dextran-conjugated drug were active in culture, the conjugates were examined in a mouse cancer model following interperitoneal injection (**Figure 2**). A more detailed and thorough study was performed with three tumor lines and reported recently [17]. The ectopic tumors grew at a much slower rate when treated with the ethanolamine-modified, methotrexate-conjugated dextrans than when treated with methotrexate alone. The methotrexate–dextran conjugate – without MMP-sensitive peptide group – had similar activity to the MMP-sensitive conjugates but the toxicity, as observed by weight loss, was significantly greater in the methotrexate–dextran conjugate than the MMP-sensitive conjugates.

4. Expert opinion

A quick search of the United States Patent and Trademark Office [27] yields at least 245 patents invented by Professor Langer. The current application seems to be a sole patent in the area of polymer therapeutics with extracellular peptide or oligosaccharide cleavage as the mechanism of activation. The use of extracellular proteases to activate drugs from polymer therapeutics has been rare although some early work in polymer therapeutics focused on extracellular proteases [28]. The claims center around the Ringsdorf model as applied to an extracellular activation, that is, digestive enzyme as described by the patent. The final claims address secondary treatments in which the secondary treatment is undefined (Claim 16) and specified as radiation (Claim 17). These

claims are not supported with evidentiary examples but these claims indicate that future work is examining the synergistic effects known to occur with many APIs and radiation therapy [29-31].

Most information that is presented in the patent as supporting examples has been published since the original patent application [14-17]. It does not appear that this therapeutic molecule has progressed to clinical trials although this cannot be ruled out at this time. Several other polymer therapeutics are at present in clinical trials [2,4] and substantiate that the drug conjugates are a viable therapeutic approach. Dextrans are widely examined [32] and are one of few polymeric carriers that have advanced to clinical trials [33] although no current product is on the market. Dextran polymers are particularly interesting owing to the extensive information gained from clinical administration over the past half-a-century [34]. It should be particularly noted, however, that although the examples cited and the chemistries described use dextran as the carrier, the claims do not specify the polymeric carrier. It is, therefore, the extracellular nature of the activation that is the essence of this novel technology.

Extracellular activation of prodrugs is not a novel in itself and the proteolytic activation used as an example, MMP-2, is no exception to investigation for prodrugs [35]. The MMPs are perceived as underutilized targets [36] for many diseases but cancer in particular. Cancer has a very active proteolytic environment that could be exploited for therapy using this mechanism [37]. MMP inhibitors have failed [38] partially owing to the homeostatic nature of the proteases [39]. Therefore, it is a much more logical approach to utilize the activity of the proteases to activate an API that has action through another molecular target. For this reason, micellar [40,41] and hydrogel-based systems [22,42-47] have been examined in recent years for therapeutic and imaging applications. This recent activity indicates that the ideas examined in this patent are significant and should be further examined for clinical applications particularly as there are many diseases that are proteolytic in nature [48-53] and adding further levels of targeting to reduce toxicity could be quite advantageous.

Acknowledgement

Our original work on protease-activated drug delivery systems has been supported by the National Institutes of Health, National Eye Institute, National Instituted for Neurologic Disorders and Stroke, American Brain Tumor Association, American Association of Colleges of Pharmacy and the American Foundation for Pharmaceutical Education.

Declaration of interest

The author states no conflict of interest and has received no payment in preparation of this manuscript.

Bibliography

1. Imming P, Sinning C, Meyer A. Drugs, their targets and the nature and number of drug targets. Nat Rev Drug Discov 2006;5(10):821-34
2. Satchi-Fainaro R, Duncan R, Barnes CM. Polymer therapeutics for cancer: current status and future challenges. Adv Polym Sci 2006;193:1-65
3. Garnett MC. Targeted drug conjugates: principles and progress. Adv Drug Deliv Rev 2001;53(2):171-216
4. Duncan R. Polymer conjugates as anticancer nanomedicines. Nat Rev Cancer 2006;6(9):688-701
5. Chabner BA, Amrein PC, Druker BJ, et al. Antineoplastic agents. 11th edition. In: Brunton LL, Lazo JS, Parker KL, editors, Goodman & Gilman's the pharmacological basis of therapeutics. McGraw-Hill: New York; 2006. xxiii, pp. 1315-1403
6. Ringsdorf H. Structure and properties of pharmacologically active polymers. J Polym Sci Polym Symp 1975;51(1):135-53
7. Duncan R. The dawning era of polymer therapeutics. Nat Rev Drug Discov 2003;2(5):347-60
8. Maeda H. The Enhanced Permeability and Retention (Epr) effect in tumor vasculature: the key role of tumor-selective macromolecular drug targeting. Adv Enzyme Regul 2001;41:189-207
9. Matsumura Y, Maeda H. A new concept for macromolecular therapeutics in cancer-chemotherapy – mechanism of tumoritropic accumulation of proteins and the antitumor agent smancs. Cancer Res 1986;46(12):6387-92
10. Jain RK. Barriers to drug-delivery in solid tumors. Sci Am 1994;271(1):58-65
11. Seymour LW, Duncan R, Strohalm J, Kopecek J. Effect of Molecular Weight (Mw) of N-(2-Hydroxypropyl) Methacrylamide copolymers on body distribution and rate of excretion after subcutaneous, intraperitoneal, and intravenous administration to rats. J Biomed Mater Res 1987;21(11):1341-58
12. Rennke HG, Venkatachalam MA. Glomerular-permeability of macromolecules – effect of molecular-configuration on the fractional clearance of uncharged dextran and neutral horseradish-peroxidase in the rat. J Clin Invest 1979;63(4):713-7
13. Duncan R. Drug polymer conjugates – potential for improved chemotherapy. Anticancer Drugs 1992;3(3):175-210
14. Chau Y, Tan FE, Langer R. Synthesis and characterization of dextran-peptide-methotrexate conjugates for tumor targeting via mediation by matrix metalloproteinase ii and matrix metalloproteinase Ix. Bioconjugate Chem 2004;15(4):931-41
15. Chau Y, Dang NM, Tan FE, Langer R. Investigation of targeting mechanism of new dextran-peptide-methotrexate conjugates using biodistribution study in matrix-metalloproteinase-overexpressing tumor xenograft model. J Pharm Sci 2006;95(3):542-51
16. Chau Y, Langer RS. Important factors in designing targeted delivery of cancer therapeutics via mmp-2 mediation. J Control Release 2003;91(1-2):239-40

17. Chau Y, Padera RF, Dang NM, Langer R. Antitumor efficacy of a novel polymer-peptide-drug conjugate in human tumor xenograft models. Int J Cancer 2006;118(6):1519-26
18. Turk BE, Huang LL, Piro ET, Cantley LC. Determination of protease cleavage site motifs using mixture- based oriented peptide libraries. Nat Biotechnol 2001;19(7):661-7
19. Netzel-Arnett S, Mallya SK, Nagase H, et al. Continuously recording fluorescent assays optimized for five human matrix metalloproteinases. Anal Biochem 1991;195(1):86-92
20. Drug Innovation & Design, Inc. Tumor protease activated prodrugs of phosphoramide mustard analogs with toxification and detoxification functionalities. US5659061; 1997
21. Timar F, Botyanszki J, Suli-Vargha H, et al. The antiproliferative action of a melphalan hexapeptide with collagenase-cleavable site. Cancer Chemother Pharmacol 1998;41(4):292-8
22. Tauro JR, Gemeinhart RA. Matrix metalloprotease triggered local delivery of cancer chemotherapeutics. Bioconjugate Chem 2005;16(5):1133-9
23. Napier University. Tumour targeting prodrugds activated by metallo matrix proteinases. WO2002072620; 2002
24. Copeland RA, Albright CF, Combs AP, et al. Peptidase-cleavable, targeted antineoplastic drugs and their therapeutic use. US6844318; 2005
25. Boehringer Ingelheim Pharma. Enzyme-activated anti-tumor prodrug compounds. US6855689; 2005
26. Massova I, Fridman R, Mobashery S. Structural insights into the catalytic domains of human matrix metalloprotease-2 and human matrix metalloprotease-9: implications for substrate specificities. J Mol Model 1997;3(1):17-30
27. The United States Patent and Trademark Office. Available form: http://www.uspto.gov/main/search.html
28. Drobnik J, Kopecek J, Labsky J, et al. Enzymatic cleavage of side-chains of synthetic water-soluble polymers. makromolekulare chemie-macromolecular chemistry and physics 1976;177(10):2833-48
29. Ke S, Milas L, Charnsangavej C, et al. Potentiation of radioresponse by polymer-drug conjugates. J Control Release 2001;74(1-3):237-42
30. Shiah JG, Sun Y, Kopeckova P, et al. Combination chemotherapy and photodynamic therapy of targetable N-(2-hydroxypropyl)methacrylamide copolymer- doxorubicin/mesochlorin E6;-Ov-Tl 16 antibody immunoconjugates. J Control Release 2001;74(1-3):249-53
31. Dubay RA, Rose PG, O'Malley DM, et al. Evaluation of concurrent and adjuvant carboplatin with radiation therapy for locally advanced cervical cancer. Gynecol Oncol 2004;94(1):121-4
32. Mehvar R. Dextrans for targeted and sustained delivery of therapeutic and imaging agents. J Control Release 2000;69(1):1-25
33. Danhauser-Riedl S, Hausmann E, Schick HD, et al. Phase-I clinical and pharmacokinetic trial of Dextran Conjugated Doxorubicin (Ad-70, Dox-Oxd). Invest New Drugs 1993;11(2-3):187-95
34. Thoren L. The dextrans-clinical data. Devel Biol Stand 1981;48:157-167
35. Kline T, Torgov MY, Mendelsohn BS, et al. Novel antitumor prodrugs designed for activation by matrix metalloproteinases-2 and -9. Mol Pharm 2004;1(1):9-22
36. Vartak D, Gemeinhart RA. Matrix metalloproteases: underutilized targets for drug delivery. J Drug Target 2007;15(1):1-21
37. Overall CM, Kleifeld O. Validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. Nat Rev Cancer 2006;6(3):227-39
38. Coussens LM, Fingleton B, Matrisian LM. Cancer therapy - matrix metalloproteinase inhibitors and cancer: trials and tribulations. Science 2002;295(5564):2387-92
39. Martin MD, Matrisian LM. The other side of mmps: protective roles in tumor progression. Cancer Metastasis Rev 2007;26(3-4):717-24
40. Bae M, Cho S, Song J, et al. Metalloprotease-specific poly(ethylene glycol) methyl ether-peptide-doxorubicin conjugate for targeting anticancer drug delivery based on angiogenesis. Drugs Exp Clin Res 2003;29(1):15-23
41. Byun Y, Kim S, Lee G, et al. Development of Mmps specific peg-peptide-doxorubicin conjugates based on angiogenesis. Eur J Cancer 2002;38:S130-1
42. Tauro JR, Gemeinhart RA. Extracellular protease activation of chemotherapeutics from hydrogel matrices: a new paradigm for local chemotherapy. Mol Pharmaceutics 2005;2(5):435-8
43. Tauro JR, Lee BS, Lateef SS, Gemeinhart RA. Matrix metalloprotease selective peptide substrates cleavage within hydrogel matrices for cancer chemotherapy activation. Peptides 2008: doi:10.1016/j.peptides.2008;.06.021
44. Mart RJ, Osborne RD, Stevens MM, Ulijn RV. Peptide-based stimuli-responsive biomaterials. Soft Matter 2006;2(10):822-35
45. Rawsterne RE, Gough JE, Rutten FJM, et al. Controlling protein retention on enzyme-responsive surfaces. Surf Interface Anal 2006;38(11):1505-11
46. Ulijn RV. Enzyme-responsive materials: a new class of smart biomaterials. J Mater Chem 2006;16(23):2217-25
47. Thornton PD, McConnell G, Ulijn RV. Enzyme responsive polymer hydrogel beads. Chem Commun 2005;47:5913-5
48. Harris ED Jr, Krane SM. An endopeptidase from rheumatoid synovial tissue culture. Biochim Biophys Acta 1972;258(2):566-76
49. Parks WC, Wilson CL, Lopez-Boado YS. Matrix metalloproteinases as modulators of inflammation and innate immunity. Nat Rev Immunol 2004;4(8):617-29
50. Kvanta A, Shen WY, Sarman S, et al. Matrix metalloproteinase (Mmp) expression in experimental choroidal neovascularization. Curr Eye Res 2000;21(3):684-90
51. Newby AC, Pauschinger M, Spinale FG. From tadpole tails to transgenic mice: metalloproteinases have brought about a metamorphosis in our understanding of cardiovascular disease. Cardiovasc Res 2006;69(3):559-561
52. Clark AW, Krekoski CA, Bou SS, et al. Increased gelatinase a (Mmp-2) and gelatinase B (Mmp-9) activities in human brain after focal ischemia. Neurosci Lett 1997;238(1-2):53-6
53. Kirkegaard T, Hansen A, Bruun E, Brynskov J. Expression and localisation of matrix metalloproteinases and their natural inhibitors in fistulae of patients with Crohn's disease. Gut 2004;53(5):701-9

Patent details

Title: Targeted Drug Delivery
Assignee: Massachusetts Institute of Technology
Inventors: Chau Y, Langer RS, Luo Y
Priority date: 03/03/2006
Filing date: 05/03/2007
Publication date: 13/09/2007
Publication no.: WO2007103364
Manuscript draft date: July 9 2008

Affiliation

Richard A Gemeinhart[1,2]
[†]Address for correspondence
[†1]Associate Professor of Pharmaceutics
and Bioengineering
University of Illinois,
College of Pharmacy,
Department of Biopharmaceutical Sciences,
833 South Wood Street (MC 865),
Chicago, IL 60612-7231, USA
Tel: +1 312 996-2253; Fax: +1 312 996 2784;
E-mail: rag@uic.edu
[2]University of Illinois,
College of Engineering,
Department of Bioengineering,
Chicago, IL 60612, USA