UNITED STATES PATENT AND TRADEMARK OFFICE

BEFORE THE PATENT TRIAL AND APPEAL BOARD

APOTEX INC.,
Petitioner,

v.

CELGENE CORPORATION,
Patent Owner

Case IPR2023-00512
Patent 8,846,628

EXPERT DECLARATION OF JAMES L. MULLINS, PH.D.

CELGENE 2149
APOTEX v. CELGENE
IPR2023-00512

I. INTRODUCTION AND ENGAGEMENT

I, Dr. James L. Mullins, hereby declare as follows:

1. I have been retained by Wilmer Cutler Pickering Hale and Dorr LLP on behalf of Celgene Corporation to offer my opinion on the authenticity of the below publications in connection with a Petition for *inter partes* review of U.S. Pat. No. 8,846,628.

2. I am being compensated at $275.00/hour, my ordinary and customary consulting rate for my work, plus reimbursement for any reasonable expenses. My compensation is based solely on the amount of time that I devote to activities related to this case and is in no way contingent on the nature of my findings, the presentation of my findings in testimony, or the outcome of this or any other proceeding. I have no other financial interest in this proceeding.

3. My opinions and the basis for my opinions are set forth below.

4. Unless otherwise noted, the statements made herein are based on my personal knowledge, and if called to testify about this declaration, I could and would do so competently and truthfully.

5. I am presently Dean Emeritus of Libraries and Esther Ellis Norton Professor Emeritus at Purdue University. My career as a professional and academic/research librarian spanned more than 44 years including library positions at Indiana University, Villanova University, Massachusetts Institute of Technology, and

Purdue University. Appendix A is a true and correct copy of my curriculum vitae describing my background and experience.

6. In 2018, I founded the firm Prior Art Documentation Librarian Services, LLC, located at 106 Berrow, Williamsburg, VA 23188 after purchasing the intellectual property of and successor to Prior Art Documentation, LLC located at 711 South Race Street, Urbana, IL 61801. Further information about my firm, Prior Art Documentation Librarian Services, LLC (PADLS), is available at www.priorartdoclib.com. Since founding Prior Art Documentation Librarian Services, LLC, in 2018, I have completed over 124 declarations for 39 law firms as of August 2023. I have been deposed in two cases one in February 2019 and a second in June 2023.

II. BACKGROUND AND QUALIFICATIONS

7. I received a Bachelor of Arts degree in History, Religion and Political Science in 1972 as well as a Master of Arts degree in Library Science in 1973 from the University of Iowa. I received my Ph.D. in Academic Library Management with an emphasis in Legal Librarianship in 1984 from Indiana University. Over forty- four years, I held various positions and served as a leader in the field of library and information sciences. Most recently, I contributed a chapter to a book to be published by the Association of College and Research Libraries (ACRL) in the Fall, 2022.

8. I am presently Dean Emeritus of Libraries and Esther Ellis Norton Professor Emeritus at Purdue University and have been since January 1, 2018. I have

been previously employed as follows:

- Dean of Libraries and Professor and Esther Ellis Norton Professor, Purdue University, West Lafayette, IN (2004-2017)
- Assistant/Associate Director for Administration, Massachusetts Institute of Technology (MIT) Libraries, Cambridge, MA (2000-2004)
- University Librarian and Director, Falvey Memorial Library, Villanova University, Villanova, PA (1996-2000)
- Director of Library Services, Indiana University South Bend, South Bend, IN (1978-1996)
- Part-time Instructor, School of Library and Information Science, Indiana University, Bloomington, IN (1979-1996)
- Associate Law Librarian, and associated titles, Indiana University School of Law, Bloomington, IN (1974-1978)
- Catalog Librarian, Assistant Professor, Georgia Southern College (now University), Statesboro, GA (1973-1974)

9. I am a member of the American Library Association ("ALA"), where I served as the chair of the Research Committee of the Association of College and Research Libraries ("ACRL"). My service to ALA included service on the editorial board of the most prominent library journal, *College and Research Libraries*. I also served on the Standards Committee, College Section of the Association of College and

Research Libraries, where I was instrumental in developing a re-issue of the *Standards for College Libraries* in 2000.

10. I am an author of numerous publications in the field of library science and have given presentations in library sciences at national and international conferences. During more than 44 years as an academic librarian and library science scholar, I have gained extensive experience with catalog records and online library management systems (LMS) built using Machine-Readable Cataloging ("MARC") standards. As an academic library administrator, I have had responsibility to ensure that students were educated to identify, locate, assess, and integrate information garnered from research library resources. I have also facilitated the research of faculty colleagues either directly or through the provision of and access to the requisite print and/or digital materials and services at the universities where I worked.

11. Based on my experience identified above and detailed in my curriculum vitae, which is attached hereto as Appendix A, I consider myself to be an expert in the field of library science and academic library administration. I have previously offered my opinions on the public availability and authenticity of documents in over 80 cases. I have been deposed in one case.

III. BACKGROUND ON PUBLIC ACCESSIBILITY

A. Scope of This Declaration

12. I am not a lawyer, and I am not rendering an opinion on the legal question of whether a particular document is, or is not, a "printed publication" under the law. I

am, however, rendering my expert opinion on the authenticity of the document referenced herein to the extent that persons interested and ordinarily skilled in the subject matter or art, exercising reasonable diligence, could have located the document.

13. I am informed by counsel that an item is considered authentic if there is sufficient evidence to support a finding that the item is what it is claimed to be. I am also informed that authenticity can be established based on the contents of the document itself, such as the appearance, content, substance, internal patterns, or other distinctive characteristics of the item.

B. Person of Ordinary Skill in the Art

14. In forming the opinions expressed in this declaration, I have reviewed the documents and appendices referenced herein. These materials are records created in the ordinary course of business by publishers, libraries, indexing services, and others. From my years of experience, I am familiar with the process for creating many of these records, and I know that these records are created by people with knowledge of the information contained within the record. Further, these records are created with the expectation that researchers and other members of the public will use them. All materials cited in this declaration and its appendices are of a type that experts in my field would reasonably rely upon and refer to in forming their opinions.

15. I have been informed by counsel that a "person of ordinary skill in the art at the time of the inventions" (POSA) is a hypothetical person who is presumed to be

familiar with the relevant field and its literature at the time of the inventions. This hypothetical person is also a person of ordinary creativity, capable of understanding the scientific principles applicable to the pertinent field.

16. I have been informed by counsel that, Petitioner's experts, Drs. Buckton and Batchelor, each put forth identical definitions of a POSA. Ex.1002(Buckton Decl.), ¶17; Ex.1003(Batchelor Decl.), ¶16. They both opine that a POSA as of December 5, 2008 "would have had (1) a Pharm.D., or a Ph.D. in pharmaceutical sciences, chemical engineering, chemistry, or related discipline; and (2) at least two to four years of experience with pharmaceutical design, formulation, development, and/or manufacturing of oral dosage forms." Ex.1002(Buckton Decl.), ¶17; Ex.1003(Batchelor Decl.), ¶16.

IV. OPINION REGARDING AUTHENTICITY

A. Document: Exhibit 2002. FDA approves Onureg® (azacitidine tablets) for acute myeloid leukemia ("FDA Approves Onureg®")

17. I have been asked to opine on, Exhibit 2002, an article published by the U.S. Food and Drug Administration, titled: *FDA approves Onureg® (azacitidine tablets) for acute myeloid leukemia* ("FDA Approves Onureg®").

18. I have evaluated FDA Approves Onureg® several ways: (1) by accessing and reviewing FDA Approves Onureg®, Exhibit 2002, provided to me by counsel; and (2) a digital copy, of FDA Approves Onureg®, Attachment A-1, accessible to me digitally on the U.S. Food and Drug Administration (FDA) website at:

https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-onureg-azacitidine-tablets-acute-myeloid-leukemia.

19. Attachment A-1 is a download I made on August 14, 2023, from the U.S. Food and Drug Administration website at: https://www.fda.gov/drugs/resources-information-approved-drugs/fda-approves-onureg-azacitidine-tablets-acute-myeloid-leukemia. I compared Attachment A-1 with Exhibit 2002 and found no difference in content.

20. Having accessed, retrieved, and downloaded FDA Approves Onureg® from the website of the U.S. Food and Drug Administration I can verify that Exhibit 2002 is an authentic document.

21. I conclude and affirm that Exhibit 2002 is an authentic document.

B. Document: Exhibit 2006. Canadian Product 200mg Onureg® Product Information

22. I have been asked to opine on, Exhibit 2006, a web page published by the Canadian government, titled: *Canadian Product 200mg Onureg® Product Information*. I have evaluated *Canadian Product 200mg Onureg® Product Information* several ways: (1) by accessing and reviewing *Canadian Product 200mg Onureg® Product Information*, Exhibit 2006, provided to me by counsel; and (2) a digital copy, of *Canadian Product 200mg Onureg® Product Information*, Attachment A-2 accessible to me digitally on the Canadian government website at: https://health-products.canada.ca/dpd-bdpp/info?lang=eng&code=99943.

23. Attachment A-2 is a download I made on August 14, 2023 from the Canadian government website at: https://health-products.canada.ca/dpd-bdpp/info?lang=eng&code=99943. I compared Attachment A-2 with Exhibit 2006 and found no difference in content except as indicated below in the next paragraph.

24. I did note that footnote #3 does not show on Ex.2006. The version now available, that I reviewed and is Attachment A-2, is designated as Version 4.0.1 with a date modified as 2023-05-25, does include footnote #3, whereas the date modified on Ex.2006 is 2023-01-24. Footnote #2 is missing from both Ex.2006 and Attachment A-2.

25. Having accessed, retrieved, and downloaded *Canadian Product 200mg Onureg® Product Information* from the website of the Canadian government I can verify that Exhibit 2006 is an authentic document.

26. I conclude and affirm that Exhibit 2006 is an authentic document.

C. Document: Exhibit 2007. Canadian Product 300mg Onureg® Product Information

27. I have been asked to opine on, Exhibit 2007, a web page published by the Canadian government, titled: *Canadian Product 300mg Onureg® Product Information*. I have evaluated *Canadian Product 300mg Onureg® Product Information* several ways: (1) by accessing and reviewing *Canadian Product 300mg Onureg® Product Information*, Exhibit 2007, provided to me by counsel; and (2) a digital copy, of *Canadian Product 300mg Onureg® Product Information*, Attachment A-3, accessible

to me digitally on the Canadian government website at: https://health-products.canada.ca/dpd-bdpp/info?lang=eng&code=99944.

28. Attachment A-3 is a download I made on August 14, 2023, from the Canadian government website at: https://health-products.canada.ca/dpd-bdpp/info?lang=eng&code=99944. I compared Attachment A-3 with Exhibit 2007 and found no difference in content, with the exception noted in the next paragraph.

29. I did note that on page 3 of Ex.2007 under the heading, "Prescription", *American Hospital Fomulary Services* (AHF): 10:00.00 does not appear. In addition Footnote #3 does not appear on Ex.2007.

30. The version now available, that I reviewed and is Attachment A-3, is designated as Version 4.0.1 with a date modified as 2023-05-25, does include *American Hospital Fomulary Services* (AHF): 10:00.00 and footnote #3, whereas the date modified on Ex.2006 is 2023-01-24. Footnote #2 is missing from both Ex.2007 and Attachment: A-3.

31. Having accessed, retrieved, and downloaded *Canadian Product 300mg Onureg® Product Information* from the website of the Canadian government I can verify that Exhibit 2007 is an authentic document.

32. I conclude and affirm that Exhibit 2007 is an authentic document.

D. Document: Exhibit 2008. Canadian Monograph Onureg®

33. I have been asked to opine on, Exhibit 2008, a web page published by the

Canadian government, titled: *Canadian Monograph Onureg®.* I have evaluated *Canadian Monograph Onureg®* several ways: (1) by accessing and reviewing *Canadian Monograph Onureg®*, Exhibit 2008, provided to me by counsel; and (2) a digital copy, of *Canadian Monograph Onureg®*, Attachment A-4, accessible to me digitally on the Canadian government website at: https://pdf.hres.ca/dpd_pm/00059490.PDF.

34. Attachment A-4 is a download I made on August 14, 2023, from the Canadian government website at: https://pdf.hres.ca/dpd_pm/00059490.PDF. I compared Attachment A-4 with Exhibit 2008 and found no difference in content.

35. Having accessed, retrieved, and downloaded *Canadian Monograph Onureg®* from the Canadian government website I can verify that Exhibit 2008 is an authentic document.

36. I conclude and affirm that Exhibit 2008 is an authentic document.

E. Document: Exhibit 2013. European Commission Decision 17.6.21

37. I have been asked to opine on, Exhibit 2013, a decision published by the European Commission, titled: *European Commission Decision 17.6.21.* I have evaluated *European Commission Decision 17.6.21* several ways: (1) by accessing and reviewing *European Commission Decision 17.6.21*, Exhibit 2013, provided to me by counsel; and (2) a digital copy, of *European Commission Decision 17.6.21*, Attachment A-5, accessible to me digitally on the European Commission website at:

https://ec.europa.eu/health/documents/community-register/2021/20210617151848/dec_151848_en.pdf.

38. Attachment A-5 is a download I made on August 14, 2023, from the European Commission website at: https://ec.europa.eu/health/documents/community-register/2021/20210617151848/dec_151848_en.pdf. I compared Attachment A-5 with Exhibit 2013 and found no difference in content.

39. Having accessed, retrieved, and downloaded *European Commission Decision 17.6.21* from the European Commission website I can verify that Exhibit 2013 is an authentic document.

40. I conclude and affirm that Exhibit 2013 is an authentic document.

F. Document: Exhibit 2016. Yvonne Perrie and Thomas Rades. *Pharmaceutical: Drug Delivery and Targeting.* FASTtrack Series; Pharmaceutical Press, 2010. 230 pages ("Perrie")

41. I have been asked to opine on Exhibit 2016, a book titled *Pharmaceutical: Drug Delivery and Targeting*, FASTtrack series, published by the Pharmaceutical Press, in 2010. ("Perrie").

42. I have evaluated Perrie several ways: (1) by accessing and reviewing Perrie, Exhibit 2016, provided to me by counsel; and (2) by retrieving and assessing scans from the print copy of Perrie owned by Purdue University Libraries. Attachment A-6 was provided to me, at my request, by the Purdue University Libraries Interlibrary Loan Department on August 16, 2023.

43. I compared Attachment A-6 with Exhibit 2016 and found no difference in content. There were two pages that were not scanned through my omission in the request and those are: “Introduction to the FASTtrack series” and “About the Authors”.

44. Having retrieved, and assessed Perrie from the Purdue University Libraries, where if it were authentic it would be held and made accessible, I can verify that Exhibit 2016 is an authentic document.

45. I conclude and affirm that Exhibit 2016 is an authentic document.

G. Document: Exhibit 2023. National Cancer Institute 1978 (“National Cancer Institute”)

46. I have been asked to opine on, Exhibit 2023, an article published by the National Institutes of Health, titled: *Bioassay of 5-azacytidine for Possible Carcinogenicity* (“National Cancer Institute”). In this declaration National Cancer Institute will indicate the article.

47. I have evaluated National Cancer Institute several ways: (1) by accessing and reviewing National Cancer Institute, Exhibit 2023, provided to me by counsel; and (2) a digital copy, of National Cancer Institute, Attachment A-7, accessible to me digitally through the National Institutes of Health website at: https://ntp.niehs.nih.gov/sites/default/files/ntp/htdocs/lt_rpts/tr042.pdf

48. Attachment A-7 is a download I made on August 14, 2023, from the National Institutes of Health website at:

https://ntp.niehs.nih.gov/sites/default/files/ntp/htdocs/lt_rpts/tr042.pdf. I compared Attachment A-7 with Exhibit 2023 and found no difference in content.

49. I did note that on Exhibit 2023 there is a label indicating that Exhibit 2023 was retrieved from a library located in Germany,: ZB MED - Informationszentrum Lebenswissenschaften, Standort Köln, as indicated below by ownership stamp and call number:



50. Having accessed, retrieved, and downloaded National Cancer Institute from the National Institutes of Health website I can verify that Exhibit 2023 is a complete excerpt from the original document and has no content differences from Attachment A-7.

51. I conclude and affirm that National Cancer Institute, Exhibit 2023 is a complete excerpt from the original document.

H. Document: Exhibit 2025. *Onureg® Label*

52. I have been asked to opine on, Exhibit 2025, a label published by the U.S. Food and Drug Administration, titled: *Onureg® Label.*

53. I have evaluated *Onureg® Label* several ways: (1) by accessing and reviewing *Onureg® Label*, Exhibit 2025, provided to me by counsel; and (2) a digital

copy, of *Onureg® Label*, Attachment A-8, accessible to me digitally through the U.S. Food and Drug Administration website at:

https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/214120s000lbl.pdf.

54. Attachment A-8 is a download I made on August 14, 2023, from the U.S. Food and Drug Administration website at:

https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/214120s000lbl.pdf.

55. I compared Attachment A-8 with Exhibit 2025 and found no difference in content.

56. Having accessed, retrieved, and downloaded *Onureg® Label* from the U.S. Food and Drug Administration website I can verify that Exhibit 2025 is an authentic document.

57. I conclude and affirm that Exhibit 2025 is an authentic document.

I. Document: Exhibit 2042. "Introduction and Perspective" by Mark Gibson published in *Pharmaceutical Preformulation and Formulation: a Practical Guide from Candidate Drug Selection to Commercial Dosage Form*, as Chapter One, edited by Mark Gibson. Interpharm Press, 2001. 596 pages, Chapter One, pages 1-11. ("Gibson")

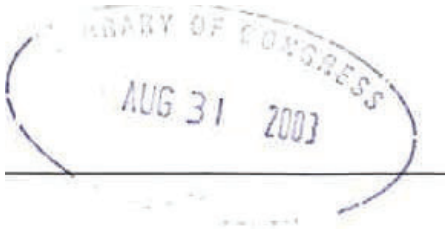
58. I have been asked to opine on, Exhibit 2042, a book published by the Interpharm Press, titled: *Pharmaceutical Preformulation and Formulation: a practical Guide from Candidate Drug Selection to Commercial Dosage Form,* Chapter One: "Introduction and Perspective" edited by Mark Gibson.

59. In this declaration Gibson will indicate the article, Chapter One:

"Introduction and Perspective" and when appropriate the entire book: *Pharmaceutical Preformulation and Formulation : a practical Guide from Candidate Drug Selection to Commercial Dosage Form* edited by Mark Gibson.

60. I have evaluated Gibson several ways: (1) by accessing and reviewing Gibson, Exhibit 2042, provided to me by counsel; and (2) a digital copy, of Gibson, Attachment A-9, provided to me on August 15, 2023 at my request by the Wisconsin TechSearch (WTS) from the print copy owned by the Library of Congress.

61. Attachment A-9 includes: cover, title page, verso of the title page (copyright) with a stamp that reads, LIBRARY OF CONGRESS AUG 31, 2001, as shown below; Contents; and Chapter 1 "Introduction and Perspective, pages 1-11, and back cover with a bar code label that reads: LIBRARY OF CONGRESS, 0 008 002 642 0.



62. I compared Gibson, Chapter 1 from Attachment A-9 with Exhibit 2042 and found no difference in content.

63. Having retrieved, and assessed Gibson from the Library of Congress, I can verify that Exhibit 2042 is a complete excerpt from the original document.

64. I conclude and affirm that Exhibit 2042 is a complete excerpt from the original document.

V. AVAILABILITY FOR CROSS-EXAMINATION

65. In signing this Declaration, I recognize that this Declaration will be filed as evidence in a contested case before the Patent Trial and Appeal Board of the U.S. Patent and Trademark Office. I also recognize that I may be subject to cross- examination in the case and that cross-examination will take place within the United States. If cross-examination is required of me, I will appear for cross-examination within the United States during the time allotted for cross-examination.

VI. RIGHT TO SUPPLEMENT

66. I reserve the right to supplement my opinions in the future to respond to any arguments that the Patent Owner raises and to take into account new information as it becomes available to me.

VII. SIGNATURE

67. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code.

68. I declare under penalty of perjury that the foregoing is true and correct. Executed on August 15, 2023.

August 17, 2023

James L. Mullins, Ph.D.

Appendix A

JAMES L. MULLINS, PhD

Prior Art Documentation Librarian Services, LLC

205 Saint Cuthbert, Williamsburg, VA 23188

jlmullins@priorartdoclib.com

ph. 765 479 4956

Prior Art Documentation Librarian Services, LLC. (PADLS). Founded January 2018. As of July, 2023, 1 have completed 123 declarations for 39 law firms; five depositions were scheduled, three cancelled a few days prior, one was held in February, 2019 and a second was held in June 2023.

Library Experience:

2018-present Dean Emeritus, Libraries & Norton Professor Emeritus, Purdue University

2011 - 2017 Dean of Libraries & Esther Ellis Norton Professor, Purdue University

2004 - 2011 Dean of Libraries & Professor, Purdue University, West Lafayette, IN

2000-2004 Assistant/Associate Director for Administration, MIT Libraries, Massachusetts Institute of Technology, Cambridge, MA.

1996-2000 University Librarian & Director, Falvey Memorial Library. Villanova University, Villanova, PA.

1978-1996 Director of Library Services, Indiana University South Bend.

1974-1978 Associate Librarian, Indiana University Bloomington, School of Law.

1973-1974 Instructor/Catalog Librarian. Georgia Southern College (now University).

Teaching Experience:

1977-1996 Associate Professor (part-time), School of Library and Information Science, Indiana University. Subjects taught: Cataloging, Management, and Academic Librarianship.

Education:

1972 - University of Iowa. Honors Bachelor of Arts, History, Religion & Political Science.

1973 - University of Iowa. Master of Arts in Library Science.

1984 - Indiana University. Doctor of Philosophy. Concentration: Academic Library Administration. Emphasis: Law Librarianship.

Awards and Recognition:

2017 Wilmeth Active Learning Center/Library of Engineering and Science, Grand Reading Room, was announced by President Mitch Daniels, Purdue University, that it would be re-named the James L. Mullins Reading Room to honor his leadership and reputation in the academic library profession. September 2017. Portrait unveiled December 2017.

2017 Distinguished Alumnus Award by the School of Informatics and Computing, Indiana University, Bloomington. Given June 25, 2017.

2016 Hugh C. Atkinson Memorial Award, jointly sponsored by the four divisions of the American Library Association (ALA), June 27, 2016.

2015 ACRL Excellence in University Libraries Award, April 23, 2015.

Named Esther Ellis Norton Professor of Library Science by Purdue Trustees, December 11, 2011.

International Review Panel to evaluate the University of Pretoria Library, February 20 – 24, 2011. Pretoria, South Africa.

Publications: (selected)

Designing the Academic Library, Chapter Contributor. Association of College and Research Libraries, American Library Association, 2022.

A Purdue Icon: Creation, Life, and Legacy, edited by James L. Mullins, Founder's Series, Purdue University Press, 138pp., August 2017.

"The policy and institutional framework." In *Research Data Management, Practical Strategies for Information Professionals,* edited by Ray, J M. Purdue University Press, pp.25-44, 2014.

"DataCite: linking research to data sets and content." In Benson, P and Silver, S. *What Editors Want: An Author's Guide to Scientific Journal Publishing*. University of Chicago Press, pp. 21-23, December 2012.

"Library Publishing Services: Strategies for Success," with R. Crow, O. Ivins, A. Mower, C. Murray-Rust, J. Ogburn, D Nesdill, M. Newton, J. Speer, C. Watkinson. *Scholarly Publishing and Academic Resources Coalition (SPARC),* version 2.0, March 2012.

"The Changing Definition and Role of Collections and Services in the University Research Library." *Indiana Libraries,* Vol 31, Number 1 (2012), pp.18-24.

"Are MLS Graduates Being Prepared for the Changing and Emerging Roles that Librarians must now assume within Research Libraries?" *Journal of Library Administration.* Volume 52, Issue 1, 2012, p. 124-132

Baykoucheva, Svetla. What Do Libraries Have to Do with e-Science?: An Interview with James L. Mullins, Dean of Purdue University Libraries. Chem. Inf. Bull. [Online] 2011, 63 (1), 45-49. http://www.acscinf.org/publications/bulletin/63-1/mullins.php (accessed Mar 16, 2011).

"The Challenges of e-Science Data-set Management and Scholarly Communication for Domain Sciences and Technology: a Role for Academic Libraries and Librarians," chapter in, *The Digital Deluge: Can Libraries Cope with e-Science?"* Deanna B. Marcum and Gerald George, editors, Libraries Unlimited/Teacher Ideas Press, 2009. (a monograph publication of the combined proceedings of the KIT/CLIR proceedings).

"Bringing Librarianship to e-Science," *College and Research Libraries.* vol. 70, no. 3, May 2009, editorial.

"The Librarian's Role in e-Science" *Joho Kanri (Journal on Information Processing and Management),* Japan Science and Technology Agency (formerly Japan Information Center of Science and Technology), Tokyo, Japan. Translated into Japanese by Taeko Kato. March 2008.

The Challenge of e-Science Data-set Management to Domain Sciences and Engineering: a Role for Academic Libraries and Librarians," KIT (Kanazawa Institute of Technology)/CLIR (Council of Library and Information Resources) International Roundtable for Library and Information Science, July 5-6, 2007. Developments in e-science status quo and the challenge, The Japan Foundation, 2007.

"An Administrative Perspective," Chapter 14, *Proven Strategies for Building an Information Literacy Program*, Susan Curzon and Lynn Lampert, editors, Neal-Schuman Publishers, Inc., New York, 2007. pp. 229-237.

Library Management and Marketing in a Multicultural World, proceedings of the IFLA Management and Marketing (M&M) Section, Shanghai, China, August 16-17, 2006, edited. K.G. Saur, Munchen, Germany, June 2007. 390 pp.

Top Ten Assumptions for the Future of Academic Libraries and Librarians: a report from the ACRL Research Committee, with Frank R. Allen and Jon R. Hufford. *College & Research Libraries,* April 2007, vol.68, no.4. pp.240-241, 246.

To Stand the Test of Time: Long-term Stewardship of Digital Data Sets in Science and Engineering. A report to the National Science Foundation from the ARL Workshop on New Collaborative Relationships: the Role of Academic Libraries in the Digital Data Universe. September 26-27, 2006, Arlington, VA. p.141. http://www.arl.org/bm~doc/digdatarpt.pdf

"Enabling Interaction and Quality in a Distributed Data DRIS," *Enabling Interaction and Quality: Beyond the Hanseatic League*. 8th International Conference on Current Research Information Systems, with D. Scott Brandt and Michael Witt. Promoted by euro CRIS. Leuven University Press, 2006. pp.55-62. Editors: Anne Garns Steine Asserson and Eduard J. Simons.

"Standards for College Libraries, the final version approved January 2000," prepared by the ACRL College Libraries Standards Committee (member), *C&RL News*, March 2000, p.175-182.

"Standards for College Libraries: a draft," prepared by the ACRL College Libraries Section, Standards Committee (member), *C&RL News*, May 1999, p. 375-381.

"Statistical Measures of Usage of Web-based Resources," *The Serials Librarian,* vol. 36, no. 1-2 (1999) p. 207-10.

"An Opportunity: Cooperation between the Library and Computer Services," in *Building Partnerships: Computing and Library Professionals.* Edited by Anne G. Lipow and Sheila D. Creth. Berkeley and San Carlos, CA, Library Solutions Press, 1995. p. 69-70.

"Faculty Status of Librarians: A Comparative Study of Two Universities in the United Kingdom and How They Compare to the Association of College and Research Libraries Standards, " in *Academic Librarianship, Past, Present, and Future: a Festschrift in Honor of David Kaser.* Englewood, Colorado; Libraries Unlimited, 1989. p. 67-78. Review in: *College & Research Libraries,* vol. 51, no. 6. November 1990, p. 573-574.

Presentations: (Representative)

"How Long the Odyssey? Transitioning the Library and Librarians to Meet the Needs and Expectations of the 21st Century University," David Kaser Lecture, School of Informatics & Computing, Indiana University, Bloomington, IN, November 16, 2015.

Presentation at University of Cape Town, Cape Town, South Africa, August 20, 2015.

"The Challenge of Discovering Science and Technology Information," Moderator, International Federation of Library Associations (IFLA) Science and Technological Libraries Section Program, Cape Town, South Africa, August 18, 2015.

"An Odyssey in Data Management: Purdue University," International Federation of Library Associations (IFLA) Research Data Management: Finding Our Role – A program of the Research Data Alliance, Cape Town, South Africa, August 17, 2015.

Presentation at University of Pretoria, Pretoria, South Africa, August 11, 2015.

Co-Convener with Sarah Thomas, Harvard University, at the Harvard Purdue Symposium on Data Management, Harvard University, Cambridge, MA, June 15-18, 2015.

"Strategic Communication," panel discussion on the Director's role and perspective on library communications at Committee on Institutional Cooperation (CIC) Center for Library Initiatives (CLI) Annual Conference, University of Illinois Urbana-Champaign, May 20, 2015.

"Issues in Data Management," panel discussion moderated by Catherine Woteki, United States Undersecretary for Research, Education & Economics at 20th Agriculture Network Information Collaborative (AgNIC) Annual Meeting in the National Agricultural Library, Beltsville, MD, May 6, 2015.

"Active learning/IMPACT & the Active Learning Center at Purdue University," Florida Institute of Technology, Melbourne, FL, February 11, 2015.

"Science+art=creativity: libraries and the new collaborative thinking," panel moderator, International Federation of Library Associations (IFLA) 80th General Conference and Assembly, Lyon, France, August 19, 2014.

"Purdue University The Active Learning Center—A new concept for a library," Association of University Architects 59th Annual National Conference, University of Notre Dame, South Bend, IN, June 23, 2014.

"Big Data & Implications for Academic Libraries," keynote speaker, Greater Western Library Alliance (GWLA) Cyber-infrastructure Conference, Kansas City, MO, May 28, 2014.

"Research Infrastructure," panel moderator, Association of Research Libraries (ARL) 164th Membership Meeting, Ohio State University, Columbus, OH, May 7, 2014.

"An Eight Year Odyssey in Data Management: Purdue University," International Association of Scientific and Technological University Libraries (IATUL) 2013 Workshop Research Data Management: Finding Our Role, University of Oxford, UK, December 2013.

"Purdue University Libraries & Press: from collaboration to integration," Ithaka Sustainable Scholarship, The Evolving Digital Landscape: New Roles and Responsibilities in Higher Education, libraries as publishers, New York, New York, October 2013.

"Tsinghua and Purdue: Research Libraries for the 21st Century," Tsinghua University, Tsinghua, China, August 2013.

"Purdue Publishing Experience in the Libraries Publishing Coalition," Association of American University Presses Annual Meeting, Press-Library Coalition Panel, Boston, Massachusetts, June 21, 2013.

"Indiana University Librarians Day: Purdue University Libraries Ready for the 21st Century," Indiana University Purdue University Indianapolis (IUPUI), June 7, 2013.

"Purdue University Libraries and Open Access; CNI Project Update," Coalition for Networked Information, San Antonio, TX, April 5, 2013.

Memorial Resolution, honoring Joseph Brannon, to the Board of the Association of College & Research Libraries, Seattle, WA, January 2013.

"An overview of sustaining e-Science collaboration in an Academic Research Library—the Purdue experience," Duraspace e-Science Institute webcast, October 17, 2012.

"The Role of Libraries in Data Curation, Access, and Preservation: an International Perspective, " Panel Moderator, 78th General Conference and Assembly, International Federation of Library Associations, Helsinki, Finland, August 15, 2012.

"21st Century Libraries," moderator of First Plenary Session, International Association of Technological University Libraries 33rd Annual Conference, Singapore, June 4, 2012.

"Planning for New Buildings on Campus," panel presenter, University of Calgary Building Symposium on Designing Libraries for the 21st Century, Calgary, Alberta, Canada, May 17, 2012.

"Data Management and e-Science, the Purdue Response." Wiley-Blackwell Executive Seminar-2012, Washington, DC, March 23, 2012.

"An overview of Sustaining e-Science Collaboration in Academic Research Libraries and the Purdue Experience." Leadership & Career Development Program Institute, Association of Research Libraries (ARL). Houston, TX, March 21, 2012.

"An overview of Data Activities at Purdue University in response to Data Management Requirements." Coalition for Academic Scientific Computation (CASC). Arlington, VA, September 8, 2011.

"Getting on Track with Tenure," Association of College and Research Libraries (ACRL) Research Program Committee. Washington, DC, June 26, 2011.

"Integration of the Press and Libraries Collaboration to Promote Scholarly Communication," Association of Library Collections & Technical Services (ALCTS) Scholarly Communication Interest Group – American Library Association, New Orleans, Louisiana, June 25, 2011.

"Cooperation for improving access to scholarly communication," with N. Lossau (Germany), C. Mazurek (Poland), J. Stokker (Australia), panel moderator and presenter, Second Plenary Session, International Association of Scientific and Technological University Libraries (IATUL) 32nd Conference 2011, Warsaw, Poland. May 29-June 2, 2011.

"Riding the Wave of Data," STM Annual Spring Conference 2011. Trailblazing & transforming scholarly publishing **2011**. Washington, D.C., April 28, 2011.

"Confronting old assumptions to assume new roles: physical and operational integration of the Press and Libraries at Purdue University," keynote speaker, 2011 BioOne Publishers & Partners Meeting. Washington, D. C., April 22, 2011.

"Are MLS Graduates Being Prepared for the Changing and Emerging Roles that Librarians must now assume within Research Libraries?" University of Oklahoma Libraries Seminar, March 4, 2011, Oklahoma City, Oklahoma.

"The Future Role of University Librarians," the University of Cape Town, South Africa, February 25, 2011.

"New Roles for Librarians: the Application of Library Science to Scientific/Technical Research – Purdue University – a case study. International Council for Science and Technology (ICSTI); Ottawa, Canada. June 9, 2009.

"Reinventing Science Librarianship: Models for the Future," Association of Research Libraries / Coalition for Networked Information. October 16-17th, 2008, Arlington, VA. Moderator and convener of Data Curation: Issues and Challenges.

"Practical Implementation and Opportunities Created at Purdue University," African Digital Curation Conference, Pretoria, South Africa, (live video transmission), February 12, 2008.

Keynote speaker. "*Scholarly Communication & Academe: The Winter of Our Discontent,"* XXVII Charleston Conference on Issues in Book and Serial Acquisition, Charleston, South Carolina. November 8, 2007.

Keynote speaker. "*Enabling Access to Scientific & Technical Data-sets in e-Science: a role for Library and Archival Sciences,"* Greater Western Library Alliance (GWLA), Tucson, Arizona. September 17, 2007. A meeting of library directors and vice presidents for research of member institutions.

"*The Challenge of e-Science Data-set Management to Domain Sciences and Engineering: a Role for Academic Libraries and Librarians,"* KIT (Kanazawa Institute of Technology)/CLIR (Council of

Library and Information Resources) International Roundtable for Library and Information Science, July 5-6, 2007. Invited to participate by the Deputy Librarian of Congress.

International Association of Technological University Libraries (IATUL), Stockholm, Sweden. June 8, 2007**.** Invited paper**,** *Enabling International Access to Scientific Data-sets: creation of the Distributed Data Curation Center (D2C2).*

"A New Collaboration for Librarians: The Principles of Library and Archival Sciences Applied to the Curation of Datasets," Symposium of the Libraries and the College of Engineering, University of Louisville, April 6, 2007.

"Purdue University Libraries: Through Pre-eminent Innovation and Creativity, Meeting the Challenges of the Information Age," Board of Trustees, Purdue University, February 15, 2007.

ARL Workshop on New Collaborative Relationships: The Role of Academic Libraries in the Digital Data Universe, September 26-27, 2006, Arlington, VA. Invited participant.

NARA and SDSC: A partnership. A panel before the National Science Foundation, June 27, 2006. Arlington, VA. Invited participant.

"Kaleidoscope of Scientific Literacy: fusing new connections," with Diane Rein, American Library Association, Association of College and Research Libraries, Science & Technology Section, Annual Conference, New Orleans, June 26th, 2006.

"Leadership for Learning: Building a Culture of Teaching in Academic Libraries – an administrative perspective," American Library Association, Association of College and Research Libraries, Instruction Section, Annual Conference, New Orleans, June 25th, 2006.

"Building an interdisciplinary research program in an academic library:
how the Libraries'associate dean for research makes a difference at Purdue University," International Association of Technological University Libraries (IATUL), Porto, Portugal, May 23rd, 2006.

"Enabling Interaction and Quality in a Distributed Data DRIS," *Enabling Interaction and Quality: Beyond the Hanseatic League*. 8th International Conference on Current Research Information Systems, with D. Scott Brandt and Michael Witt. Promoted by euro CRIS, Bergen, Norway, May 12th, 2006, Brandt, and Witt presented in person

"Interdisciplinary Research," with D. Scott Brandt, Coalition for Networked Information (CNI) Spring Meeting: Project Briefing, Washington, D.C., April 3rd, 2006.

"An Interview with Purdue's James Mullins," a podcast submitted by Matt Pasiewicz, on *Educause Connect,* http://connect.educause.edu/James_L_Mullins_Interview_CNI_2005

"Managing Long-Lived Digital Data-sets and their Curation: Interdisciplinary Policy Issues," Managing Digital Assets Forum, Association of Research Libraries (ARL), Washington, D.C., October 28th, 2005.
"The Odyssey of a Librarian." Indiana Library Federation (ILF), District 2 Meeting, South Bend, Indiana. October 4th, 2005.
"New College Library Standards," Standards Committee Presentation, ALA, Chicago, July 7, 2000.
SUNY Library Directors, Lake George, New York. "*The College Library Standards: a Tool for Assessment."* April 5, 2000.
Tri-State College Library Association, *Finding You Have Talents You Never Knew You Had,* Penn State Great Valley, March 25, 2000.
Using Web Statistics, American Library Association, New Orleans, June 24, 1999.
Keynote speaker at the JSTOR Workshop, January 29, 30, 1999. University of Pennsylvania, Philadelphia, PA.
"The New Standards for Electronic Resources Statistics," Society of Scholarly Publishers, Washington, D.C., September 17, 1998.
"Evaluating Online Resources: Now that you've got them what do you do?," joint presenter with Chuck Hamaker, LSU, at the NASIG Conference, Boulder, Colorado. June 1998.
"What Employers Are Looking for in New Librarians?" Pennsylvania Library Association, Philadelphia. September 26, 1997.
"The Theory of Matrix Management" panel presentation of the Comparative Library Organization Committee of the Library Organization and Management Section of the Library Administration and Management Association, a division of the American Library Association, Annual Meeting, Chicago, June 24, 1990.

Professional Involvement: (summary of recent emphasis)

The focus for my professional involvement and research has moved recently toward managing massive data-sets. This has resulted in working with faculty in the sciences and technology to determine how librarians can collaborate in managing, curating, and preserving data-sets for future access and documentation. This has included various speaking opportunities as well as participation in planning with the National Science Foundation (NSF) on ways in which librarians can be integrated more completely into the funded research process. Participation in the Kanazawa Institute of Technology/Council of Library Resources Roundtable was particularly rewarding and provided new opportunities to share with international colleagues the issues surrounding data-set management. I was

the champion for the creation of the Distributed Data Curation Center (D2C2) at Purdue University (http://d2c2.lib.purdue.edu/)

Throughout my career, beginning with my dissertation, I have been actively involved with assessing and evaluating libraries. In the fall of 1999, I contacted twenty-two academic library directors to determine whether the need was also felt by others. The response was overwhelmingly affirmative. This resulted in a meeting at ALA Midwinter, January 2000. A formal meeting followed at Villanova University in April 2000. As convener, I helped to form the University Libraries Group (ULG), modeled after the Oberlin Group for college libraries. The ULG is made up of university libraries that support diverse wide-ranging programs through doctoral level and have a level of support that places them in the top tier of academic institutions. A few of the member libraries, along with Villanova, are William and Mary, Wake Forest, Lehigh, Carnegie-Mellon, Tufts, Marquette, Miami of Ohio, and Southern Methodist.

In 1994 appointed to the Standards Committee, College Section, Association of College and Research Libraries. During the next six years, the Committee concentrated on changing the focus of the standards from quantitative analysis of input and output factors to emphasis on assessment of the outcome. Culmination of the work was a re-issue of the *Standards for College Libraries* in 2000. The knowledge gained through my work experience enabled me to formulate the changes needed in the standards. This work allowed for close collaboration with accrediting agencies, both professional and regional.

During this same time another focus emerged, the impact of digital resources. Through my work on the JSTOR Statistics Task Force, standards were developed on the collection of use of electronic databases. This Standard was later adopted in 1998 by the International Consortium of Library Consortia (ICOLC).

In 2002, the American Library Association appointed me to serve as the liaison to the Marketing and Management Section of the International Federation of Library Associations (IFLA).

Professional Service: (representative list)

Nominations Committee, Association of Research Libraries (ARL), 2016.

Steering Committee, Scholarly Publishing and Academic Resources Coalition (SPARC), 2016 – 2017.

"Excellence in Library Services," Chair, Review Team, University of Hong Kong, Hong Kong, August 24-27, 2015.

Chair, Management Advisory Board, 2015-2017; Member, Scientific Advisory Board, arXiv, Cornell University, 1/1/2013 – present.

Advisory Board for the Wayne State University School of Library and Information Science, July 2012 – present.

Advisory Board for Microsoft Academic Search, 2012 – 2015. Redmond, WA.
Transforming Research Libraries, a Strategic Direction Steering Committee of the Association of Research Libraries (ARL), 2012-2015.
Science and Technology section, representing ARL, International Federation of Library Associations (IFLA), Chair, 2013 – 2017; Member, 2011 to present.
Member of University of Pretoria, South Africa, Library Review Committee. August 2013.
Co-chair, Local Arrangements Planning Committee for 2013 Conference, Association of College and Research Libraries (ACRL), a division of the American Library Association (ALA).
Association of Research Libraries Leadership & Career Development Program Mentor, 2011-2017.
e-Science Task Force, Association of Research Libraries. July 2006 – present. Chair, October 2011 – October 2012.
Board of Directors, International Association of Technological University Libraries (IATUL). January 2008 – December 2014.
Midwest Collaborative for Library Services (MCLS); Board Member, October 2010 – December 2012.
Chair, Library Directors, Committee on Institutional Cooperation (CIC), July 2010 – June 2012.
Board of Directors, Association of Research Libraries (ARL); October 2008 – October 2011.
Scholarly Communication Steering Committee, Association of Research Libraries (ARL) 2008-2011.
Editorial Board, *College and Research Libraries*, Association of College and Research Libraries, American Library Association. January 2008 – December 2014.
Chair, Organizing Committee for IATUL Conference 2010, June 21-24, 2010, Purdue University, West Lafayette, Indiana/Chicago, Illinois.
Conference Planning Committee for National Conference of the Association of College and Research Libraries, 2009, Seattle, Washington.
Research Committee, Association of College and Research Libraries, ACRL, division of ALA. 2002-2007, chair, 2005-2007.
Association of Research Libraries, Search and Screen Committee, Executive Director. March – January 2008.
Center for Research Libraries, Board of Directors. April 2006 – April 2012.
Academic Libraries of Indiana, Board of Directors, 2004 – present. Vice-president, 2005-2007. President, 2007- 2009.

ALA Representative to the International Federation of Library Associations (IFLA), Marketing and Management (M&M) Section, initial term 2003-2007, re-appointed for second term, 2007-2011.
Invited to represent Research Libraries at the ACRL/3M Wonewok Retreat to assess Marketing of Academic Libraries, October 2002.
Hugh A. Atkinson Award Committee, LAMA Representative, ALA, 2001-2005.
Program Committee, Library Administrators and Management Association (LAMA), a division of ALA. 1996-2001.
ACRL, Standards and Accreditation Committee, a division of ALA. Liaison to RBMS Section of ACRL. 1997-2002.
Elected to the Executive Committee of LAMA, LOMS, a division of the American Library Association, 1998-2000. Nominated as Chair/Elect for 2003 – 2005.
Columbia University Press Advisory Committee. 1996 - 2000.
LITA/LAMA Conference Evaluation Committee, Pittsburgh, Pennsylvania, October 1996.
"New Learning Communities," Coalition for Networked Information, Indianapolis. November 19-21, 1995. Facilitator for invitational, national conference committed to developing collaborative learning and teaching techniques, involving librarians.
Planning Committee-Evaluation. LITA/LAMA 1996 Conference, Pittsburgh. This first conference, to be held jointly between two divisions of ALA, will focus on new technologies within libraries.
Indiana Cooperative Library Services Authority (InCoLSA), elected to Executive Committee, April 1991, served as President in 1993-94. InCoLSA is a statewide network of academic, public, school, and special libraries that supports library cooperation for cataloging, interlibrary loan, collection development and application of new technologies.
Governor's Conference on Libraries and Information Services. Served on Planning Committee, Academic Libraries Representative, appointed by the Governor to represent academic libraries in Indiana, Chair, Finance Committee, April 1989-July 1991.
Indiana Library Endowment Foundation Board, 1984-92. Charter Member, 1984, President, 1988-1992. 2004-2005.

University Service: (Summary)

Served on search and screen committees for senior positions including chancellor, dean, and directors; most recently I have been asked to serve on the search committee for the provost of Purdue University. At MIT service included the Library Council & appointment to the Administrative Council by President

Vest, 2001-2003 & Member of the Faculty Committee on the Library System. At Purdue appointed by the President to the Search Committee for the Provost, October 2007 to May 2008; member of the Capital Projects Committee, and IT Operational Oversight Committee as senior academic dean, 2008-2014.

Global Council, Global Policy Institute, 2012 – 2016.

Academic Program Excellence and Rankings (APER) project team, 2014.

Representative of the Academic Deans on the Re-engineering Business Operations, Purdue University, 2016 –

Academic Deans Council chaired by Provost – 2004 – 2017.

University Promotion and Tenure Committee – 2006 – 2017.

"Outstanding Team Award, Electronic Reserve Project," served as Chair, recognition awarded by the President of Villanova University to one team who made an outstanding contribution to the operations of the University, selected by a committee of administrators, faculty, and staff. Awarded September 9, 1999.

Nominated for the IUSB Lundquist Award, 1995 & 1996. The Lundquist award is given to faculty who have "exhibited excellence in teaching, scholarly or artistic achievement, and diversified relevant service..."

Attachment A-1

FDA approves Onureg (azacitidine tablets) for acute myeloid leukemia

On September 1, 2020, the Food and Drug Administration approved azacitidine tablets (ONUREG®, Celgene Corporation) for continued treatment of patients with acute myeloid leukemia who achieved first complete remission (CR) or complete remission with incomplete blood count recovery (CRi) following intensive induction chemotherapy and are not able to complete intensive curative therapy.

Efficacy was investigated in QUAZAR (NCT01757535), a multicenter, randomized, double-blind, placebo-controlled trial. Patients (n=472) who achieved CR or CRi with intensive induction chemotherapy with or without receiving subsequent consolidation therapy were randomized 1:1 to receive Onureg 300 mg (n=238) or placebo (n=234) orally on days 1 to 14 of each 28-day cycle.

The main efficacy outcome measure was overall survival (OS). Median OS was 24.7 months (95% CI: 18.7, 30.5) in the Onureg arm and 14.8 months (95% CI: 11.7, 17.6) in the placebo arm (HR 0.69; 95% CI: 0.55, 0.86; p=0.0009). A subgroup analysis showed consistency in the OS benefit for patients in either CR or CRi.

Adverse reactions in ≥ 10% patients receiving Onureg were nausea, vomiting, diarrhea, fatigue/asthenia, constipation, pneumonia, abdominal pain, arthralgia, decreased appetite, febrile neutropenia, dizziness, and pain in extremity.

The recommended Onureg dose is 300 mg orally once daily with or without food on days 1 through 14 of each 28-day cycle. Continue Onureg until disease progression or unacceptable toxicity.

View full prescribing information for ONUREG (https://www.accessdata.fda.gov/drugsatfda_docs/label/2020/214120s000lbl.pdf).

This application was granted priority review and Onureg was granted orphan drug designation. A description of FDA expedited programs is in the Guidance for Industry: Expedited Programs for Serious Conditions-Drugs and Biologics (/regulatory-information/search-fda-guidance-documents/expedited-programs-serious-conditions-drugs-and-biologics).

Healthcare professionals should report all serious adverse events suspected to be associated with the use of any medicine and device to FDA's MedWatch Reporting System

(https://www.accessdata.fda.gov/scripts/medwatch/index.cfm) or by calling 1-800-FDA-1088.

For assistance with single-patient INDs for investigational oncology products, healthcare professionals may contact OCE's Project Facilitate (/about-fda/oncology-center-excellence/project-facilitate) at 240-402-0004 or email OncProjectFacilitate@fda.hhs.gov (mailto:OncProjectFacilitate@fda.hhs.gov).

For information on the COVID-19 pandemic, see the following resources:

- FDA: Coronavirus Disease 2019 (COVID-19)
- NCI: Coronavirus: What People With Cancer Should Know (https://www.cancer.gov/contact/emergency-preparedness/coronavirus)
- CDC: Coronavirus (COVID-19) (https://www.cdc.gov/coronavirus/2019-ncov/index.html)

Follow the Oncology Center of Excellence (/about-fda/fda-organization/oncology-center-excellence) on Twitter @FDAOncology (http://www.twitter.com/@fdaoncology) (http://www.fda.gov/about-fda/website-policies/website-disclaimer).

Attachment A-2

Government of Canada Gouvernement du Canada

Product information

From Health Canada

New search

The product monograph is developed by a drug sponsor according to guidelines published by Health Canada that provide direction on the content and format. The veterinary labelling is developed by the drug sponsor according to the Food and Drug Regulations. While Health Canada reviews the product monograph or the veterinary labelling as part of the drug review process, it remains the responsibility of the drug sponsor to ensure that the product monograph or the veterinary labelling is complete and accurate.

Current status:

Marketed

Current status date:

2021-03-10

Original market date: [1]

2021-03-10

Product name:

ONUREG

Help on accessing alternative formats, such as Portable Document Format (PDF), Microsoft Word and PowerPoint (PPT) files, can be obtained in the alternate format help section.

DIN:

02510197

Product Monograph/Veterinary Labelling:

Date: 2021-01-04

Product monograph/Veterinary Labelling (PDF version ~ 175K)

Company:

CELGENE INC
300 2344 Alfred-Nobel Boulevard
Saint-Laurent
Quebec
Canada H4S 0A4

Class:

Human

Dosage form(s):

Tablet

Route(s) of administration:

Oral

Number of active ingredient(s):

1

Schedule(s):

Prescription

American Hospital Formulary Service (AHFS): [3]

10:00.00

Anatomical Therapeutic Chemical (ATC): [4]

L01BC07 AZACITIDINE

Active ingredient group (AIG) number: [5]

0152665002

List of active ingredient(s)

Active ingredient(s)	Strength
AZACITIDINE	200 MG

New search | Same active ingredient group number

Footnotes

1 The earliest marketed date recorded in the Drug Product Database.

3 The American Hospital Formulary Service permits an easy review of information on a group of drugs with similar activities and uses and allows the reader to determine quickly the similarities and differences among drugs within a group. *AHFS® Pharmacologic/Therapeutic Classification©* used with permission. © 2022, the American Society of Health-System Pharmacists, Inc. (ASHP). The Data is a part of the AHFS Drug Information®; ASHP is not responsible for the accuracy of transpositions from the original context.

4 The purpose of the World Health Organization (WHO) Anatomical Therapeutic Chemical (ATC) classification system is to be used as a tool for drug utilization research in order to improve quality of drug use. Drugs are divided into different groups according to the organ or system on which they act and their chemical, pharmacological and therapeutical properties.

5 The AIG number is a 10 digit number that identifies products that have the same active ingredient(s) and ingredient strength(s). The AIG is comprised of three portions:

- the first portion (2 digits) identifies the number of active ingredients,
- the second portion (5 digits) identifies the unique groups of active ingredients(s),
- the last portion (3 digits) identifies the active ingredient group strength. The strength group has a tolerance of -2% to +10%.

Application information

Search tips

Drug product database terminology

Drug product database data extracts

Related information

MedEffect Canada

Adverse drug reaction - veterinary drugs

Notice of compliance database

Licensed natural health products database

Contact us

Content support

Technical support

Version 4.0.1

Date modified: 2023-05-25

Attachment A-3

Government of Canada Gouvernement du Canada

Product information

From Health Canada

New search

The product monograph is developed by a drug sponsor according to guidelines published by Health Canada that provide direction on the content and format. The veterinary labelling is developed by the drug sponsor according to the Food and Drug Regulations. While Health Canada reviews the product monograph or the veterinary labelling as part of the drug review process, it remains the responsibility of the drug sponsor to ensure that the product monograph or the veterinary labelling is complete and accurate.

Current status:

Marketed

Current status date:

2021-03-10

Original market date: [1]

2021-03-10

Product name:

ONUREG

Help on accessing alternative formats, such as Portable Document Format (PDF), Microsoft Word and PowerPoint (PPT) files, can be obtained in the alternate format help section.

DIN:

02510200

Product Monograph/Veterinary Labelling:

Date: 2021-01-04

Product monograph/Veterinary Labelling (PDF version ~ 175K)

Company:

CELGENE INC
300 2344 Alfred-Nobel Boulevard
Saint-Laurent
Quebec
Canada H4S 0A4

Class:

Human

Dosage form(s):

Tablet

Route(s) of administration:

Oral

Number of active ingredient(s):

1

Schedule(s):

Prescription

American Hospital Formulary Service (AHFS): [3]

10:00.00

Anatomical Therapeutic Chemical (ATC): [4]

L01BC07 AZACITIDINE

Active ingredient group (AIG) number: [5]

0152665003

List of active ingredient(s)

Active ingredient(s)	Strength
AZACITIDINE	300 MG

New search | Same active ingredient group number

Footnotes

1 The earliest marketed date recorded in the Drug Product Database.

3 The American Hospital Formulary Service permits an easy review of information on a group of drugs with similar activities and uses and allows the reader to determine quickly the similarities and differences among drugs within a group. *AHFS® Pharmacologic/Therapeutic Classification©* used with permission. © 2022, the American Society of Health-System Pharmacists, Inc. (ASHP). The Data is a part of the AHFS Drug Information®; ASHP is not responsible for the accuracy of transpositions from the original context.

4 The purpose of the World Health Organization (WHO) Anatomical Therapeutic Chemical (ATC) classification system is to be used as a tool for drug utilization research in order to improve quality of drug use. Drugs are divided into different groups according to the organ or system on which they act and their chemical, pharmacological and therapeutical properties.

5 The AIG number is a 10 digit number that identifies products that have the same active ingredient(s) and ingredient strength(s). The AIG is comprised of three portions:

- the first portion (2 digits) identifies the number of active ingredients,
- the second portion (5 digits) identifies the unique groups of active ingredients(s),
- the last portion (3 digits) identifies the active ingredient group strength. The strength group has a tolerance of -2% to +10%.

Application information

Search tips

Drug product database terminology

Drug product database data extracts

Related information

MedEffect Canada

Adverse drug reaction - veterinary drugs

Notice of compliance database

Licensed natural health products database

Contact us

Content support

Technical support

Version 4.0.1

Date modified: 2023-05-25

Attachment A-4

PRODUCT MONOGRAPH

INCLUDING PATIENT MEDICATION INFORMATION

PrONUREG®

azacitidine tablets

Tablets, 200 mg, 300 mg azacitidine, Oral

Antineoplastic / Pyrimidine Analogue

Celgene Inc., a Bristol-Myers Squibb company
2344 Alfred-Nobel Blvd.
Suite 300
Saint-Laurent, QC
H4S 0A4

Date of Initial Approval:
JAN-04-2021

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Submission Control No: 240668

RECENT MAJOR LABEL CHANGES

Not Applicable.

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RECENT MAJOR LABEL CHANGES 2

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PART I: HEALTH PROFESSIONAL INFORMATION

1 INDICATIONS

ONUREG® (azacitidine tablets) is a nucleoside metabolic inhibitor indicated for:

- maintenance therapy in adult patients with acute myeloid leukemia (AML) who achieved complete remission (CR) or complete remission with incomplete blood count recovery (CRi) following induction therapy with or without consolidation treatment, and who are not eligible for hematopoietic stem cell transplantation (HSCT).

Limitations of Use:

- ONUREG® is not interchangeable with, and should not be substituted with or for, azacitidine for injection. See **DOSAGE AND ADMINISTRATION, Administration** and **WARNINGS AND PRECAUTIONS, General**.
- The safety and effectiveness of ONUREG for treatment of myelodysplastic syndromes have not been established. Treatment of patients with myelodysplastic syndromes with ONUREG is not recommended outside of controlled trials. See **WARNINGS AND PRECAUTIONS, General**.

1.1 Pediatrics

Pediatrics (**< 18 years of age)**: No data are available to Health Canada; therefore, Health Canada has not authorized an indication for pediatric use.

1.2 Geriatrics

Geriatrics (≥ 65 years of age): No dosage adjustment is required for ONUREG® based on age, see **ACTION AND CLINICAL PHARMACOLOGY, Pharmacokinetics.**

2 CONTRAINDICATIONS

ONUREG® is contraindicated in patients who are hypersensitive to this drug or to any ingredient in the formulation, including any non-medicinal ingredient, or component of the container. For a complete listing, see **DOSAGE FORMS, STRENGTHS, COMPOSITION AND PACKAGING.**

ONUREG® is contraindicated in patients with advanced malignant hepatic tumors.

4 DOSAGE AND ADMINISTRATION

4.1 Dosing Considerations

- Health Canada has not authorized an indication for pediatric use, see **INDICATIONS**.
- No specific dose adjustments are recommended for elderly patients (≥ 65 years of age), see **INDICATIONS**.
- Administer an antiemetic 30 minutes prior to each dose of ONUREG for the first 2 cycles. Antiemetic prophylaxis may be omitted after 2 cycles if there has been no nausea and vomiting.

- ONUREG® can be administered to patients with renal impairment without initial dose adjustment, see **DOSAGE AND ADMINISTRATION, Recommended Dose and Dosage Adjustment** and **CLINICAL PHARMACOLOGY, Pharmacokinetics**.

4.2 Recommended Dose and Dosage Adjustment

Recommended Starting Dosage:

The recommended starting dose of ONUREG® is 300 mg orally once daily on Day 1 through Day 14 of repeated 28-day treatment cycles.

If the absolute neutrophil count (ANC) is less than 500/mcL on Day 1 of a cycle, do not administer ONUREG®. Delay the start of the cycle until the ANC is 500/mcL or more.

ONUREG® maintenance therapy should be initiated after achievement of a CR/CRi following completion of induction and consolidation therapy or following induction if consolidation therapy is not planned.

Dose Modifications During Treatment:

Dose Adjustment for Renal Impairment: No dose adjustment is required for patients with mild to moderate renal impairment.

ONUREG® can be administered to patients with severe renal impairment without initial dose adjustment. Monitor patients with severe renal impairment (creatinine clearance [CLcr] 15 to 29 mL/min calculated by Cockcroft-Gault formula) more frequently for adverse reactions and modify the ONUREG dosage for adverse reactions, see **WARNINGS AND PRECAUTIONS, Monitoring and Laboratory Tests**.

Dose Adjustment for Hepatic Impairment: ONUREG® has not been studied in patients with pre-existing severe hepatic impairment (total bilirubin > 3 × ULN).

A recommended dosage of ONUREG® has not been established for patients with moderate hepatic impairment (total bilirubin > 1.5 to 3 × ULN).

No dose adjustment of ONUREG® is recommended for patients with mild hepatic impairment (total bilirubin ≤ ULN and AST > ULN, or total bilirubin 1 to 1.5 × ULN and any AST).

Dose Adjustment for Adverse Reactions: Dose modification guidelines for hematologic and non-hematologic adverse reactions are recommended based on clinical and laboratory findings if toxicities are judged related to ONUREG® (see Table 1).

Table 1: Dose Adjustment for Hematological and Nonhematological Adverse Reactions

Adverse Reaction	Recommended Action
Grade 4 Neutropenia	First Occurrence

Adverse Reaction	Recommended Action
(Absolute Neutrophil Count (ANC): < 500/mcL) **or** **Grade 3 Neutropenia with Fever** **(ANC: 500 – 1000/mcL)**	• Interrupt dose. Resume at the same dose once neutrophils return to Grade 2 or lower. • The use of supportive care such as granulocyte colony stimulating factor (GCSF), as clinically indicated, may be considered. Occurrence in 2 Consecutive Cycles • Interrupt dose. After neutrophils return to Grade 2 or lower, reduce dose to 200 mg. • If a patient continues to experience the toxicity after dose reduction, reduce the treatment duration by 7 days. • If the toxicity continues or reoccurs after dose and schedule reduction, discontinue ONUREG®. • The use of supportive care such as GCSF, as clinically indicated, may be considered.
Grade 4 Thrombocytopenia **(Platelets: < 25,000/mcL)** **or** **Grade 3 Thrombocytopenia with Active Bleeding** **(Platelets: 25,000 – 50,000/mcL)**	First Occurrence • Interrupt dose. Resume at the same dose once platelets return to Grade 2 or lower. Occurrence in 2 Consecutive Cycles • Interrupt dose. After platelets return to Grade 2 or lower, reduce dose to 200 mg. • If a patient continues to experience the toxicity after dose reduction, reduce the treatment duration by 7 days. • If the toxicity continues or reoccurs after dose and schedule reduction, discontinue ONUREG®.
Grade 3 or Higher **Nausea, Vomiting or Diarrhea**	• Interrupt dose. Resume at the same dose once toxicity has resolved to Grade 1 or lower. • If event reoccurs, interrupt dose until resolved to Grade 1 or lower. Reduce the dose to 200 mg. • If a patient continues to experience the toxicity after dose reduction, reduce the treatment duration by 7 days. • If the toxicity continues or reoccurs after dose and schedule reduction, discontinue ONUREG®.

Adverse Reaction	Recommended Action
Other Grade 3 or Higher Nonhematologic Events	• Interrupt dose and provide medical support. Resume at the same dose once toxicity has resolved to Grade 1 or lower. • If event reoccurs, interrupt dose until resolved to Grade 1 or lower. Reduce the dose to 200 mg. • If a patient continues to experience the toxicity after dose reduction, reduce the treatment duration by 7 days. • If the toxicity continues or reoccurs after dose and schedule reduction, discontinue ONUREG®.

Continuation and Discontinuation Recommendations:

ONUREG®, given Day 1 through Day 14 of repeated 28-day treatment cycles, is intended for continuous use. ONUREG® treatment should be continued until more than 15% blasts are observed in peripheral blood or bone marrow or until unacceptable toxicity occurs. Discontinue ONUREG® if more than 15% blasts are observed in either the peripheral blood or bone marrow or at the physician's discretion.

4.3 Administration

ONUREG® can be taken with or without food. Do not split, crush, or chew ONUREG® tablets. Administer a dose at about the same time each day. Consider providing prophylactic anti-emetic therapy during ONUREG® treatment.

ONUREG® is not interchangeable with, and should not be substituted with or for, azacitidine for injection. Due to differences in exposure, the dose and schedule recommendations for ONUREG® are different from those for injectable azacitidine. Verify drug name, dose, and administration route. See **INDICATIONS, Limitation of Use.**

4.5 Missed Dose

If a dose of ONUREG® is missed, or not taken at the usual time, administer the dose as soon as possible on the same day, and return to the normal time of dose administration the following day. Do not take 2 doses on the same day. If a dose is vomited, do not take another dose on the same day, but return to the normal time of dose administration the following day.

5 OVERDOSAGE

In the event of overdose, monitor the patient with appropriate blood counts and provide supportive treatment, as necessary. There is no known specific antidote for ONUREG® overdose.

For management of a suspected drug overdose, contact your regional poison control centre.

6 DOSAGE FORMS, STRENGTHS, COMPOSITION AND PACKAGING

Table 2: Dosage Forms, Strengths, Composition and Packaging.

Route of Administration	Dosage Form / Strength/ Composition	Non-medicinal Ingredients
Oral	Tablet 200 mg azacitidine	croscarmellose sodium, hypromellose, iron oxide red, lactose monohydrate, polyethylene glycol/macrogol, magnesium stearate, mannitol, silicified microcrystalline cellulose, titanium dioxide, triacetin
Oral	Tablet 300 mg azacitidine	black iron oxide, croscarmellose sodium, hypromellose, iron oxide red, iron oxide yellow, lactose monohydrate, polyethylene glycol/macrogol, magnesium stearate, mannitol, silicified microcrystalline cellulose, titanium dioxide, triacetin

ONUREG® tablets are available in 7 count blister packages.

7 WARNINGS AND PRECAUTIONS

General

Due to substantial differences in the pharmacokinetic parameters (see **ACTION AND CLINICAL PHARMACOLOGY, Pharmacokinetics***)*, the recommended dose and schedule for ONUREG are different from those for the intravenous or subcutaneous azacitidine products. Treatment of patients using intravenous or subcutaneous azacitidine at the recommended dosage of ONUREG® may result in a fatal adverse reaction. Treatment of patients using ONUREG® at the doses recommended for intravenous or subcutaneous azacitidine may not be effective. Do not substitute ONUREG® for intravenous or subcutaneous azacitidine (see **DOSAGE AND ADMINISTRATION, Administration** and **INDICATIONS, Limitation of Uses**).

In AZA-MDS-003 (NCT01566695), 216 patients with red blood cell transfusion-dependent anemia and thrombocytopenia due to myelodysplastic syndromes were randomized to ONUREG® or placebo. One-hundred and seven patients received a median of 5 cycles of ONUREG® 300 mg daily for 21 days of a 28-day cycle. Enrollment was discontinued early due to a higher incidence of early fatal and/or serious adverse reactions in patients who received ONUREG® compared with placebo. The most frequent fatal adverse reaction was sepsis. Therefore, the safety and effectiveness of ONUREG® for treatment of myelodysplastic syndromes have not been established. Treatment of patients with myelodysplastic syndromes with ONUREG® is not recommended outside of controlled trials. (See **INDICATIONS, Limitation of Uses)**

Carcinogenesis and Mutagenesis

In vitro studies demonstrated that azacitidine is mutagenic and clastogenic in bacterial and mammalian cell systems. Azacitidine induced neoplastic lesions and tumours in multiple tissues

in rats and mice administered with azacitidine intraperitoneally (see **NON-CLINICAL TOXICOLOGY**).

Cardiovascular

Patients with a history of severe congestive heart failure, clinically unstable cardiac disease or pulmonary disease were excluded from the pivotal clinical study and therefore the safety and efficacy of ONUREG® in these patients have not been established.

No thorough clinical QT/QTc study or *in vitro* studies (hERG, canine Purkinje fiber assay) were performed to rule out the effect of ONUREG® on QT prolongation. An *in vivo* safety pharmacology study in dogs receiving azacitidine reported increased QTc interval, but interpretation of this study is limited by confounding effects associated with toxicity (see **NON-CLINICAL TOXICOLOGY**).

Driving and Operating Machinery

No studies on the effects on the ability to drive or use machinery have been performed. Patients should be advised that they may experience undesirable effects such as fatigue, asthenia, and gastrointestinal reactions such as nausea, vomiting, diarrhea and constipation, during treatment with ONUREG®. Therefore, caution should be exercised when driving or operating a vehicle or potentially dangerous machinery.

Gastrointestinal

Gastrointestinal toxicities were the most frequent adverse reactions in the ONUREG® treatment group. Nausea (64.8%), vomiting (59.7%), and diarrhea (50.4%) were reported in patients treated with ONUREG®. Grade 3 or 4 diarrhea, vomiting, or nausea occurred in 5.1%, 3.0%, and 2.5%, respectively in patients treated with ONUREG®. The first occurrence of Grade 3 or 4 diarrhea, vomiting, or nausea occurred within the first 2 cycles in 1.3%, 3.0%, and 1.7%, respectively in patients treated with ONUREG®.

Consider providing prophylactic anti-emetic therapy during ONUREG® treatment. Treat diarrhea with antidiarrheal medications promptly at the onset of symptoms.

Hematologic

New or worsening Grade 3 or higher neutropenia (41.1%), thrombocytopenia (22.5%), or febrile neutropenia (11.4%) were commonly reported (10% or more) adverse reactions in patients treated with ONUREG®. The first occurrence of Grade 3 or 4 neutropenia, thrombocytopenia, or febrile neutropenia occurred within the first 2 cycles in 19.9%, 10.6%, and 1.7%, respectively in patients treated with ONUREG®.

Monitor complete blood counts and modify the dosage as recommended (see **WARNINGS AND PRECAUTIONS, Monitoring and Laboratory Tests**). Consider the use of supportive care such as granulocyte colony stimulating factor (GCSF) as clinically indicated.

Hepatic/Biliary/Pancreatic

Patients with extensive tumor burden due to metastatic disease have been rarely reported to experience progressive hepatic coma and death during injectable treatment with azacitidine, especially in such patients with baseline serum albumin < 30 g/L. ONUREG® is contraindicated in patients with advanced malignant hepatic tumors (see **CONTRAINDICATIONS**).

Monitoring and Laboratory Tests
Complete blood count monitoring is recommended every other week for the first 2 cycles (56 days), every other week for the next 2 cycles after dose adjustment, and monthly thereafter, prior to start of next cycle.

Monitor patients with severe renal impairment (CLcr 15 to 29 mL/min) more frequently for adverse reactions and modify the ONUREG® dosage for adverse reactions, see **DOSING AND ADMINISTRATION, Recommended Dose and Dosage Adjustment.** No dose adjustment of ONUREG® is required for patients with mild to severe renal impairment (CLcr 15 to 89 mL/min), see **ACTION AND CLINICAL PHARMACOLOGY, Pharmacokinetics.**

Sexual Health

Fertility

There are no human data on the effect of azacitidine on fertility. In animals, adverse effects of azacitidine on male and female fertility have been documented. See **NON-CLINICAL TOXICOLOGY**

Reproduction

Pregnancy testing is recommended for females of reproductive potential before starting ONUREG®.

If either a female of reproductive potential wishes to have a child or a male wishes to conceive a child, they should seek advice for reproductive counseling and cryo-conservation of either ovum or sperm prior to starting ONUREG®.

7.1 Special Populations

7.1.1 Pregnant Women

Azacitidine may cause fetal harm when administered to a pregnant woman based on its mechanism of action and findings in animals. There are no available data on ONUREG® use in pregnant women. Azacitidine was teratogenic in animals and caused embryo-fetal lethality in animals at doses less than the recommended human daily dose of oral azacitidine, see **NON-CLINICAL TOXICOLOGY**.

Females of childbearing potential should be advised to avoid pregnancy during treatment with azacitidine. If this drug is used during pregnancy or if a patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. Advise females of reproductive potential to use effective contraception during treatment with ONUREG® and for at least 6 months after the last dose.

7.1.1.1 Males with Female Sexual Partners of Reproductive Potential

Males with female sexual partners of reproductive potential should not conceive a child and should use effective contraception during treatment with ONUREG®, and for at least 6 months after the last dose.

7.1.2 Breast-feeding

It is not known whether azacitidine or its metabolites are excreted in human milk or the effects on the nursing child or milk production. Due to the potential serious adverse reactions in the nursing child, breast-feeding must be discontinued during ONUREG® therapy and for one week after the last dose.

7.1.3 Pediatrics

Pediatrics (**< 18 years of age)**: No data are available to Health Canada; therefore, Health Canada has not authorized an indication for pediatric use.

7.1.4 Geriatrics

Geriatrics (≥ 65 years of age): No dosage adjustment is required for ONUREG® based on age, see **ACTION AND CLINICAL PHARMACOLOGY, Pharmacokinetics**.

8 ADVERSE REACTIONS

8.1 Adverse Reaction Overview

This section describes the safety data from the QUAZAR (CC-486-AML-001) trial (see **CLINICAL TRIALS**).

The most frequently reported adverse events (≥ 10%) were nausea, vomiting, diarrhea, neutropenia, fatigue/asthenia, anemia, constipation, thrombocytopenia, abdominal pain, respiratory tract infection, arthralgia, decreased appetite, febrile neutropenia, back pain, leukopenia, pain in extremity, dizziness and pneumonia. The most frequent serious adverse events to ONUREG® that occurred in ≥ 2% of patients were febrile neutropenia (6.8%), pneumonia (5.1%) and pyrexia (2.1%).

Permanent discontinuation of ONUREG® due to an adverse event occurred in 6.8% of patients. The most common adverse events to ONUREG® requiring permanent discontinuation in 1% or more of patients included nausea (2.1%), diarrhea (1.7%), and vomiting (1.3%).

Adverse events in the ONUREG® arm requiring dosage interruption in 2% or more of patients included neutropenia (19.9%), thrombocytopenia (8.5%), nausea (5.5%), diarrhea (4.2%), vomiting (3.8%), pneumonia (3.4%), leukopenia (2.5%), febrile neutropenia (2.1%), and alanine aminotransferase increased (2.1%). Adverse events to ONUREG® requiring dosage reduction in 1% or more of patients included neutropenia (5.5%), diarrhea (3.4%), thrombocytopenia (1.7%), and nausea (1.7%).

8.2 Clinical Trial Adverse Reactions

Because clinical trials are conducted under very specific conditions, the adverse reaction rates

observed in the clinical trials may not reflect the rates observed in practice and should not be compared to the rates in the clinical trials of another drug. Adverse reaction information from clinical trials is useful for identifying drug-related adverse events and for approximating rates.

Treatment emergent adverse events (TEAEs) observed in the QUAZAR (CC-486-AML-001) trial are listed in Table 3. Median treatment duration differed between the two groups: of 11.6 months (range: 0.5 to 74.3 months) for the ONUREG® treated patients and 5.7 months (range: 0.7 to 68.5 months) for the placebo arm.

Table 3: All Treatment Emergent Adverse Events Reported in ≥ 5% and Grade 3/4 Treatment Emergent Adverse Events Reported in ≥ 1% of ONUREG® Treated Patients (≥ 1% frequency versus placebo) from the CC-486-AML-001 trial (safety population)

System Organ Class Preferred Term	ONUREG® n = 236		Placebo n = 233	
	All Grade N (%)	Grade 3-4 N (%)	All Grade N (%)	Grade 3-4 N (%)
Gastrointestinal Disorders				
Nausea	153 (65)	6 (3)	55 (24)	1 (0.4)
Vomiting	141 (60)	7 (3.0)	23 (10)	0 (0)
Diarrhea	119 (50)	12 (5)	50 (22)	3 (1)
Constipation	91 (39)	3 (1)	56 (24)	0 (0)
Abdominal pain*	51 (22)	4 (2)	30 (13)	1 (0.4)
Flatulence	13 (6)	0 (0)	4 (2)	0 (0)
Blood and lymphatic system disorders				
Neutropenia	105 (45)	97 (41)	61 (26)	55 (24)
Thrombocytopenia	79 (34)	53 (23)	63 (27)	50 (22)
Anemia	48 (20)	33 (14)	42 (18)	30 (13)
Febrile neutropenia	28 (12)	27 (11)	18 (8)	18 (8)
Leukopenia	25 (11)	18 (8)	19 (8)	14 (6)
Infections and infestations				
Influenza	18 (8)	3 (1)	7 (3)	0 (0)
Pneumonia†	24 (10)	2 (1)	13 (6)	1 (0.4)
Urinary tract infection‡	22 (9)	4 (2)	17 (7)	2 (1)
Bronchitis	13 (6)	0 (0)	9 (4)	0 (0)
Rhinitis	12 (5)	0 (0)	4 (2)	0 (0)
Cellulitis	9 (4)	4 (2)	3 (1)	1 (0.4)
Lung Infection	4 (2)	4 (2)	2 (1)	1 (0.4)

System Organ Class Preferred Term	ONUREG® n = 236		Placebo n = 233	
	All Grade N (%)	Grade 3-4 N (%)	All Grade N (%)	Grade 3-4 N (%)
General disorders and administrative site conditions				
Fatigue / asthenia	104 (44)	9 (4)	58 (25)	3 (1)
Metabolism and nutrition disorders				
Decreased appetite	30 (13)	2 (1)	15 (6)	2 (1)
Musculoskeletal and connective tissue disorders				
Arthralgia	32 (14)	2 (1)	24 (10)	1 (0.4)
Back pain	28 (12)	3 (1)	23 (10)	2 (1)
Pain in extremity	25 (11)	1 (0.4)	12 (5)	0 (0.0)
Nervous System Disorders				
Dizziness	25 (11)	0 (0.0)	21 (9)	0 (0.0)
Syncope	5 (2)	5 (2)	1 (0.4)	1 (0.4)
Psychiatric Disorders				
Anxiety	16 (7)	0 (0.0)	8 (3)	1 (0.4)
Investigations				
Alanine aminotransferase increased	12 (5)	3 (1)	4 (2)	2 (1)
Blood Uric Acid Increased	5 (2)	5 (2)	2 (1)	2 (1)
Injury, Poisoning and Procedural Complications				
Fall	12 (5)	1 (0.4)	4 (2)	0 (0.0)
Cardiac Disorders				
Atrial Fibrillation	4 (2)	4 (2)	4 (2)	0 (0.0)

Treatment-emergent adverse events include adverse events that started between the first dose date and the date 28 days after the last dose date of study treatment.
A subject is counted only once for multiple events within preferred term/system organ class.
* Grouped terms included abdominal pain, abdominal pain upper, abdominal discomfort, and gastrointestinal pain.
† Grouped terms included pneumonia, bronchopulmonary aspergillosis, lung infection, Pneumocystis jirovecii pneumonia, atypical pneumonia, pneumonia bacterial, and pneumonia fungal.
‡ Grouped terms included urinary tract infection, urinary tract infection bacterial, Escherichia urinary tract infection, and cystitis.

Data cut-off date: 15 Jul 2019

8.3 Less Common Clinical Trial Adverse Reactions

Other adverse reactions that did not meet criteria for inclusion in Table 3 were respiratory tract infections (RTI; 18%) and weight decreased (4%) in patients treated with ONUREG®. RTI was a grouped term which included upper respiratory tract infection, respiratory tract infection, and respiratory tract infection viral.

8.4 Abnormal Laboratory Findings: Hematologic, Clinical Chemistry and Other Quantitative Data

Changes in selected laboratory abnormalities that have worsened from baseline in ≥20% of patients receiving ONUREG® with a difference versus placebo of >10% is listed in Table 4.

Table 4: Selected Laboratory Abnormalities that Worsened from Baseline Reported in ≥20% of ONUREG® Treated Patients (> 10% frequency versus placebo) from the CC-486-AML-001 trial

Laboratory Parameter[1]	ONUREG® N = 236 n (%)		Placebo N = 233 n (%)	
	All Grades	Grade ≥3	All Grades	Grade ≥3
Decrease in neutrophils	203 (86)	152 (64)	176 (76)	111 (48)
Decrease in white blood cells	201 (85)	127 (54)	165 (71)	75 (32)
Decrease in red blood cells	103 (44)	27 (11)	67 (29)	14 (6)

[1]Not all lab shifts were reported as Treatment Emergent Adverse Events (TEAEs)

8.5 Post-Market Adverse Drug Reactions

The following adverse reactions have been identified during post-approval use of intravenous or subcutaneous azacitidine.

Blood and lymphatic system disorders:

- Hemorrhagic diathesis

Cardiac disorders:

- Atrial fibrillation, cardiac failure congestive, cardiac failure, pericardial effusion

Gastrointestinal disorders:

- Colitis, intestinal perforation, pancreatitis acute, subileus

Hepatobiliary disorders:

- Hepatic failure, hepatitis, ascites, hyperbilirubinemia, jaundice

Infections and infestations:

- Sepsis, septic shock, infection, bacterial sepsis, abscess intestinal, cellulitis, pseudomonal sepsis, lower respiratory tract infection, bronchopulmonary aspergillosis, Clostridium difficile colitis, lobar pneumonia, lung infection pseudomonal

Injury, poisoning and procedural complications:

- Splenic rupture

Investigations:
- Blood creatinine increased, blood bilirubin increased, AST increased, ALT increased

Metabolism and nutrition disorders:
- Dehydration, hyperglycemia, hyponatremia, tumor lysis syndrome

Nervous system disorders:
- Grand mal convulsion

Renal and urinary disorders:
- Renal failure acute, renal failure

Respiratory, thoracic, and mediastinal disorders:
- Interstitial lung disease, pulmonary embolism, acute respiratory distress syndrome

Skin and subcutaneous tissue disorders:
Leukocytoclastic vasculitis, pyoderma gangrenosum, Sweet's syndrome (acute febrile neutrophilic dermatosis), necrotizing fasciitis

9 DRUG INTERACTIONS

9.2 Overview

Based on *in vitro* data, azacitidine metabolism does not appear to be mediated by cytochrome P450 isoforms (CYPs). Therefore, interactions with CYP inhibitors and inducers are unlikely to have any impact on the metabolism of ONUREG®.

ONUREG® is not a substrate of P-glycoprotein (P-gp).

Multiple nucleoside transporters are involved in azacitidine transport, therefore azacitidine uptake is unlikely to be altered by single nucleotide polymorphisms in individual nucleoside transporters or nucleoside modulators.

9.3 Drug-Drug Interactions

Oral azacitidine exposure was minimally affected when co-administered with a proton pump inhibitor (omeprazole). Therefore, a dose modification is not required when ONUREG® is co-administered with proton pump inhibitors or other pH modifiers.

No clinically relevant drug-drug interactions would be expected when ONUREG® is co-administered with CYP or transporter substrates. In vitro, azacitidine did not inhibit P-glycoprotein (P-gp), breast cancer resistance protein (BCRP), organic anion transporters (OAT) OAT1 and OAT3, organic anion transporting polypeptides (OATP) OATP1B1 and OATP1B3, or organic cation transporter (OCT) OCT2.

In vitro studies indicated that at concentrations up to 100 µM (approximately 30-fold higher than clinically achievable concentrations), azacitidine did not induce cytochrome P450 isoenzymes (CYPs) 1A2, 2C19, or 3A4 or 3A5. Using in vitro studies at concentrations up to 100 µM,

azacitidine did not inhibit P450 isoenzymes CYP 1A2, 2B6, 2C8, 2C9, 2C19, 2D6, 3A4, and 2E1. Therefore, CYP induction or inhibition by azacitidine at clinically achievable plasma concentrations is unlikely.

9.4 Drug-Food Interactions

The impact of food on the exposure of ONUREG® was minimal. Therefore, ONUREG® can be administered either with or without food. See **ACTION AND CLINICAL PHARMACOLOGY, Pharmacokinetics**.

9.5 Drug-Herb Interactions

No formal drug-herb interaction studies have been conducted.

9.6 Drug-Laboratory Test Interactions

No interactions identified.

9.7 Drug-Lifestyle Interactions

No formal drug-lifestyle (i.e. smoking) studies have been conducted.

10 ACTION AND CLINICAL PHARMACOLOGY

10.1 Mechanism of Action

Azacitidine is a DNA methyltransferase inhibitor and epigenetic modifier. Azacitidine is a pyrimidine nucleoside analog of cytidine that is incorporated into DNA and RNA following cellular uptake and enzymatic biotransformation to nucleotide triphosphates.

Incorporation of azacitidine into the DNA of cancer cells *in vitro*, including acute myeloid leukemia cells, inhibited DNA methyltransferases, reduced DNA methylation, and altered of gene expression, including re-expression of genes regulating tumor suppression, and cell differentiation. Incorporation of azacitidine into the RNA of cancer cells, including leukemic cells, inhibited RNA methyltransferase, reduced RNA methylation, decreased RNA stability, and decreased protein synthesis.

Anti-leukemic activity of azacitidine was demonstrated by reduction of cell viability and induction of apoptosis in AML cell lines *in vitro. In vivo*, azacitidine decreased tumor burden and increased survival in leukemic tumor models.

10.2 Pharmacodynamics

The epigenetic regulatory effect of oral azacitidine on DNA global methylation reduction in the blood was sustained with prolonged exposure of 300 mg daily administered for 14 or 21 days of a 28-day cycle in myeloid cancers including AML patients from a Phase 1/2 study. A positive correlation was observed between azacitidine plasma exposure and the pharmacodynamic effect of reduction in global DNA methylation in blood.

10.3 Pharmacokinetics

Table 5: Summary of ONUREG® Pharmacokinetic Parameters in Adult Cancer Patients after 300 mg dose

	C_{max} (ng/mL)	T_{max} (h)	$t_{½}$ (h)	$AUC_{0-\infty}$ (ng·h/mL)	CL/F (L/h)	Vz/F (L)
Single dose mean*	145.1	1.0	0.5	241.6	1242	881.1

*The geometric mean value is shown for all parameters except for T_{max} which is the median.
N = 30

$AUC_{0-\infty}$ = area under the concentration-time curve from time zero to infinity; C_{max} = maximum concentration for the first dose; CL/F = apparent clearance; $t_{1/2}$ = elimination half-life; T_{max} = time to reach C_{max}; Vz/F = apparent volume of distribution

Absorption: The geometric mean (coefficient of variation [%CV]) C_{max} and AUC values after oral administration of a 300 mg single dose were 145.1 ng/mL (63.7) and 241.6 ng h/mL (64.5), respectively. Exposure was generally linear with dose-proportional increases in systemic exposure; high intersubject variability was observed. Absorption of azacitidine was rapid, with a median T_{max} of 1-hour post-dose.

Multiple dosing at the recommended dose regimen did not result in drug accumulation.

Mean oral bioavailability relative to subcutaneous (SC) administration was approximately 11%.

Food Effect: Co-administration of a high-fat, high-calorie meal (approximately 800 to 1000 calories with 150 calories from protein, 250 calories from carbohydrate, and 500 to 600 calories from fat) with a single 300mg dose of azacitidine in adult cancer patients delayed T_{max} by approximately 1 hour and decreased C_{max} by 21%. There was no significant impact of a high-fat, high-calorie meal on azacitidine exposure (AUC_T) when compared to administrations under fasting conditions. ONUREG® may be administered with or without food.

Distribution: Following oral administration, the geometric mean apparent volume of distribution is 881 L. The plasma protein binding of azacitidine was approximately 6 to 12%.

Metabolism: Based on *in vitro* data, azacitidine metabolism does not appear to be mediated by cytochrome P450 isoenzymes (CYPs).

Metabolism of azacitidine is by spontaneous hydrolysis and deamination mediated by cytidine deaminase.

Elimination: The geometric mean apparent clearance of azacitidine was 1242 L/hour and the geometric mean half-life was approximately 0.5 hours.

Following intravenous administration of ^{14}C azacitidine to 5 cancer patients, the cumulative urinary excretion was 85% of the radioactive dose. Fecal excretion accounted for <1% of administered radioactivity over 3 days. Mean excretion of radioactivity in urine following subcutaneous administration of ^{14}C-azacitidine was 50%. The amount of unchanged azacitidine recovered in urine relative to dose was <2% following either SC or oral administration.

Special Populations and Conditions

Pediatrics: No data are available on administration of ONUREG® to pediatric or adolescent patients (< 18 years of age).

Geriatrics: Age (46 to 93 years) did not have clinically meaningful effects on the pharmacokinetics of oral azacitidine. Therefore, dose modification for ONUREG® is not required based on age.

Sex: Gender did not have clinically meaningful effects on the pharmacokinetics of azacitidine.

Ethnic origin: The effects of race (White [92%]) on the pharmacokinetics of azacitidine were not conclusive due to the small number of nonwhite patients enrolled.

Hepatic Insufficiency: The effects of moderate to severe hepatic impairment on the pharmacokinetics of azacitidine have not been studied.

Mild hepatic impairment (total bilirubin ≤ ULN and AST > ULN, or total bilirubin 1 to 1.5 x ULN and any AST) had no clinically meaningful effect on the pharmacokinetics of azacitidine.

Renal Insufficiency: In patients with cancer, the pharmacokinetics of azacitidine in 6 patients with normal renal function (CLcr >80 mL/min) and 6 patients with severe renal impairment (CLcr <30 mL/min) were compared following daily subcutaneous dosing (Days 1 through 5) at 75 mg/m2/day. Severe renal impairment increased azacitidine exposure by approximately 70% after single and 41% after multiple subcutaneous administrations.

The exposure of azacitidine (AUC) is approximately 75% lower after oral administration relative to the exposure achieved following SC administration; therefore, an increase in exposure of approximately 40% following oral administration is still considered safe and tolerable. ONUREG® can be administered to patients with renal impairment without initial dose adjustment. Patients with severe renal impairment should be closely monitored for toxicity since ONUREG® and/or its metabolites are primarily excreted through the kidney (see **DOSAGE AND ADMINISTRATION**, **Dose Modifications During Treatment)**.

Obesity: Body weight (39.3 kg to 129 kg) did not have clinically meaningful effects on the pharmacokinetics of azacitidine.

11 STORAGE, STABILITY AND DISPOSAL

Store blisters at 15º to 30ºC. Store in the original aluminum-aluminum blisters.

12 SPECIAL HANDLING INSTRUCTIONS

Do not crush tablets.

If powder comes in contact with skin, immediately and thoroughly wash with soap and water. If powder comes in contact with mucous membranes, immediately flush the area with water.

PART II: SCIENTIFIC INFORMATION

13 PHARMACEUTICAL INFORMATION

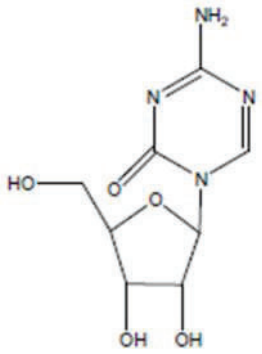
Drug Substance

Proper/Common name: azacitidine

Chemical name: 4-amino-1-β-D-ribofuranosyl-1,3,5-triazin-2(1H)-one

Molecular formula and molecular mass: $C_8H_{12}N_4O_5$, 244 g/mol

Structural formula:



Physicochemical properties: Azacitidine is a white to off-white solid. It is insoluble in acetone, ethanol, and methyl ethyl ketone; slightly soluble in ethanol/water (50/50), propylene glycol, and polyethylene glycol; sparingly soluble in water, water saturated octanol, 5% dextrose in water, N-methyl-2- pyrrolidone, normal saline and 5% Tween 80 in water; and soluble in dimethylsulfoxide (DMSO).

14 CLINICAL TRIALS

The clinical efficacy and safety of ONUREG® were evaluated in adult patients in AML remission in the CC-486-AML-001 trial.

14.1 Trial Design and Study Demographics

Table 6: Summary of Clinical Trials in Patients in AML Remission

Study # Trial Design	Dosage, route of administration and duration	Number of Subjects
CC-486-AML-001 (QUAZAR) Phase 3, double-blind, randomized, placebo-controlled, multicenter study to compare the efficacy and safety of ONUREG® plus BSC to placebo plus BSC as maintenance therapy in subjects with AML who have achieved CR or CRi following induction therapy with or without consolidation therapy.	300 mg ONUREG® or matching placebo QD for 14 days of each 28-day treatment cycle [a].	ONUREG® arm = 238 Placebo arm = 234

AML=Acute Myelogenous Leukemia, BSC = best supportive care CR=Morphologic Complete Remission, CRi=Morphologic complete remission with incomplete blood count recovery, QD = once daily
[a] Dose modification was permitted for adverse events. In the event of disease relapse (5% to 15% blasts in peripheral blood or bone marrow) dose schedule extension was permitted at investigator discretion.

CC-486-AML-001 Trial:

Adult patients aged ≥ 55 years with de novo AML, AML secondary to prior diagnosis of myelodysplastic syndromes (MDS), or chronic myelomonocytic leukemia (CMML), who had achieved first CR /CRi within 4 months +/- 7days after intensive induction chemotherapy with or without consolidation prior to randomization, were enrolled. Patients were not eligible for transplant at the time of randomization. Ineligibility to HSCT was due to age, comorbidities, performance status, no available donor, unfavourable cytogenetics or patient choice.

Patients in both treatment arms received best supportive care as deemed necessary by the investigator. Best supportive care included but was not limited to, treatment with red blood cell (RBC) transfusions; platelet transfusions; use of erythropoiesis stimulating agent; antibiotic, antiviral and/or antifungal therapy; granulocyte colony stimulating factors; anti-emetic therapy; and nutritional support.

The median treatment duration in the CC-486-AML-001 trial was 11.6 months (range: 0.5 to 74.3 months) for ONUREG® versus 5.7 months (range: 0.7 to 68.5 months) for placebo.

Table 7 summarizes the patients baseline demographic and disease-related characteristics.

Table 7: Summary of Baseline Demographics and Disease-Related Characteristics of Patients in the Phase 3 CC-486-AML-001 trial

Parameter	ONUREG® (N=238)	Placebo (N = 234)
Age (years)		
Median (Min, Max)	68.0 (55, 86)	68.0 (55, 82)
Race, n (%)		

Parameter	ONUREG® (N=238)	Placebo (N = 234)
White	216 (90.8)	197 (84.2)
Black or African American	2 (0.8)	6 (2.6)
Asian	6 (2.5)	20 (8.5)
Other	12 (5.0)	11 (4.7)
Not Collected or Reported	2 (0.8)	0
Type of AML, n (%)		
Primary (de novo)	213 (89.5)	216 (92.3)
Secondary	25 (10.5)	18 (7.7)
ECOG Performance Status, n (%)		
Grade 0	116 (48.7)	111 (47.4)
Grade 1	101 (42.4)	106 (45.3)
Grade 2	21 (8.8)	15 (6.4)
Grade 3	0	2 (0.9)
Cytogenetic Risk Status at Diagnosis, n (%)		
Intermediate[1]	203 (85.3)	203 (86.8)
Poor[2]	35 (14.7)	31 (13.2)
Received Consolidation Therapy following Induction Therapy, n (%)		
Yes	186 (78.2)	192 (82.1)
1 Cycle	110 (46.2)	102 (43.6)
2 Cycles	70 (29.4)	77 (32.9)
3 Cycles	6 (2.5)	13 (5.6)
No	52 (21.8)	42 (17.9)
MRD Status at Randomization,[3] n (%)		
Negative	133 (55.9)	111 (47.4)
Positive	103 (43.3)	116 (49.6)
Missing	2 (0.8)	7 (3.0)

AML=Acute Myelogenous Leukemia, ECOG=Eastern Cooperative Oncology Group, CR=Morphologic Complete Remission, CRi=Morphologic complete remission with incomplete blood count recovery

[1] Intermediate risk was defined as normal cytogenetics +8, t(9;11), or Other undefined.
[2] Poor risk was defined as Complex (≥ 3 abnormalities): -5; 5q-; -7; 7q-; 11q23 - non t(9;11); inv(3); t(3;3); t(6;9); or t(9;22).
[3] MRD status in bone marrow was measured during screening period by flow cytometric assay at a sensitivity level of 0.1%.

14.2 Study Results

The efficacy of ONUREG® in adult patients in AML remission was established based on the primary endpoint of overall survival (OS).

The efficacy results are summarized in Table 8. The median OS was significantly longer with ONUREG® versus placebo: 24.7 months versus 14.8 months [HR 0.69 (95% CI: 0.55, 0.86); p=0.0009], indicating a 31% reduction in the risk of death for the ONUREG® arm. The Kaplan-Meier curve displays the OS (Figure 1) results.

Table 8: Efficacy Results of the CC-486-AML-001 Trial in Patients in AML Remission

Efficacy Endpoint Statistic	**ONUREG® N = 238[d]**	**Placebo N = 234[d]**
Overall Survival (primary endpoint)		
Number of deaths, n (%)	158 (66.4)	171 (73.1)
Median overall survival (95% CI)[a] months	24.7 (18.7, 30.5)	14.8 (11.7, 17.6)
Hazard ratio$_{C/P}$ (95% CI)[b]	0.69 (0.55, 0.86)	
Stratified log-rank test: p-value [c]	0.0009	
Survival Estimates		
1-year (95% CI)	0.73 (0.67, 0.78)	0.56 (0.49, 0.62)
2-year (95% CI)	0.51 (0.44, 0.57)	0.37 (0.31, 0.43)

CI = confidence interval

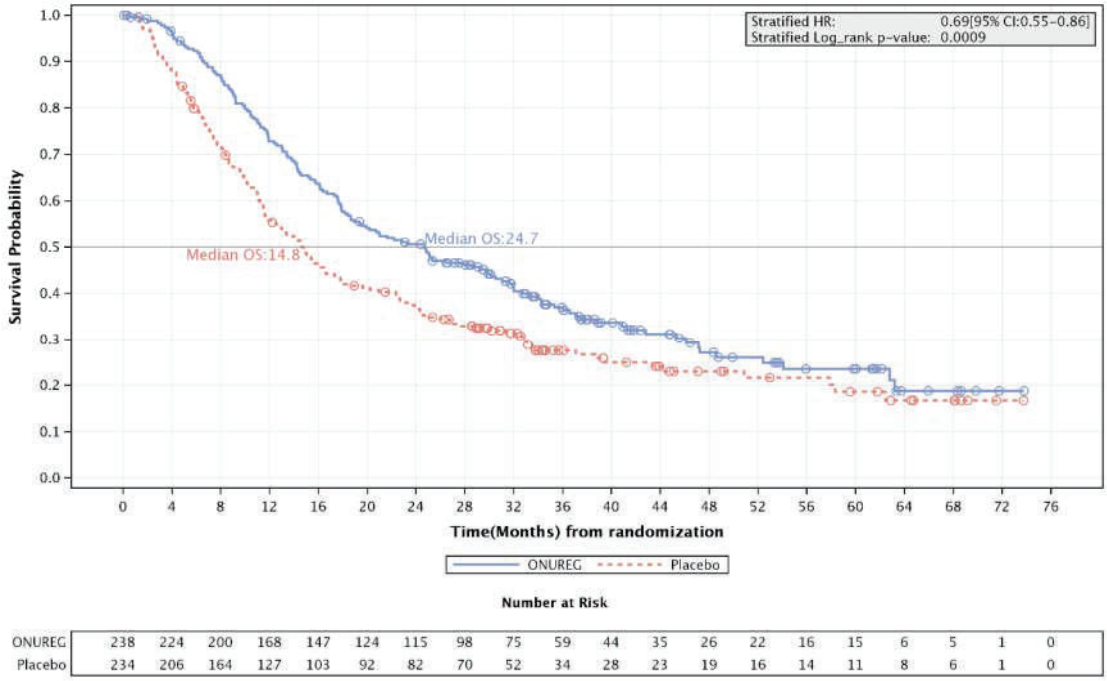
[a] Median estimate of OS is from an unstratified Kaplan-Meier analysis.

[b] The hazard ratio is from a Cox proportional hazards model stratified by age, cytogenetic risk category, and received consolidation therapy or not.

[c] The p-value is 2-sided from a log-rank test stratified by age, cytogenetic risk category, and received consolidation therapy or not.

[d] Includes patients who had dose schedule extension due to disease relapse (5% to 15% blasts in peripheral blood or bone marrow) at investigator discretion.

Figure 1: Kaplan-Meier Plot of Overall Survival for ONUREG® (CC-486) vs. Placebo



CI = confidence interval; HR = hazard ratio; OS = overall survival.

Relapse-free survival (RFS), the key secondary endpoint in the QUAZAR trial, supports the OS results. The median RFS was 10.2 months for ONUREG® versus 4.8 months for placebo [HR 0.65 (95% CI: 0.52, 0.81); p=0.0001].

Prespecified subgroup analyses of OS and RFS showed a consistent treatment effect for ONUREG® across demographic and disease-related subgroups including baseline cytogenetic risk, the number of prior consolidation cycles received, and CR/CRi status at randomization.

Using the FACIT-fatigue scale and the EQ-5D-3L health-related quality of life (HRQoL) measurements, there were no clinically meaningful differences in QoL observed between the ONUREG® + BSC and placebo + BSC treatment arms.

16 NON-CLINICAL TOXICOLOGY

Toxicology data were collected from *in vitro* studies and studies conducted in mice, rats, dogs, and rhesus monkeys. Toxicity including effects on carcinogenicity, reproductive and developmental toxicity occurred in animals at doses lower than the maximum recommended clinical dose of 300 mg of ONUREG® (equivalent to 185 mg/m^2 for a 60 kg person).

General Toxicology

Carcinogenicity:

Carcinogenicity studies have shown that azacitidine is a carcinogen in mice and rats after IP administration. The interpretation of study results is compromised by high toxicity and early mortality in mice and rats at high doses. In Sprague-Dawley rats administered 2.6 mg/kg or 5.2 mg/kg (15.6 mg/m^2 or 31.2 mg/m^2; approximately 8 to 32% of the recommended human daily dose of ONUREG) of azacitidine for approximately 9 months (34 weeks), neoplastic lesions were noted in multiple tissues, including an increased incidence of testicular tumours compared with controls. Azacitidine induced tumors of the hematopoietic system in female mice at 2.2 mg/kg (6.6 mg/m^2, approximately 4% of the recommended human daily dose of oral azacitidine on a mg/m^2 basis) administered intraperitoneally 3 times per week for 52 weeks and tumours in the lymphoreticular system, lung, mammary gland, and skin in mice treated with intraperitoneal azacitidine at 2 mg/kg (6 mg/m^2, approximately 3% of the recommended human daily dose of oral azacitidine on a mg/m^2 basis) once a week for 50 weeks.

Genotoxicity:

Genotoxicity studies conducted *in vitro* have consistently shown that azacitidine is both mutagenic and clastogenic and induces chromosome aberrations *in vitro*. Azacitidine caused a mutagenic response in bacterial systems at 1-10 µg/plate, and induced an increased number of micronuclei in mammalian cells at 0.1 to 5 µM.

Repeat-Dose:

In a 14-day oral toxicity study in dogs, mortality occurred at doses of 0.4 and 0.8 mg/kg/day (8 and 16 mg/m^2/day). Dose-related findings were present at ≥ 0.2 mg/kg/day (4 mg/m^2/day), including pancytopenia correlated with bone marrow hypoplasia, lymphoid depletion in the thymus, spleen, and lymph nodes, gland/lumen dilation and single cell necrosis in mucosal

crypts of small and large intestines and/or centrilobular hepatocellular vacuolation were observed. At 0.2 mg/kg/day, these findings were partially or completely resolved after 3 weeks of recovery period. Following parenteral azacitidine administrations at comparable dose ranges, mortality and similar target organ toxicities were observed in rodents, dogs and monkeys.

Reproductive and Developmental Toxicology:

In fertility studies in male rats, azacitidine treatment resulted in reduced fertility after IP administration of 5 mg/kg (30 mg/m^2) 3 times per week for 11 weeks before mating. Males treated with 2.5 mg/kg (15 mg/m^2) were fertile but mating with untreated females resulted in increases in preimplantation embryo loss and increases in the average number of abnormal embryos.

In mice, a 44% frequency of intrauterine embryonal death (increased absorption) were observed after a single intraperitoneal (IP) injection of 6mg/m^2 azacitidine (approximately 3.2% of the recommended human daily dose of ONUREG® on a mg/m^2 basis) on gestation day 10. Developmental abnormalities in the brain have been detected in mice given azacitidine on or before gestation day 15.

In rats, azacitidine was embryotoxic when given IP on gestation days 4-8 (postimplantation) at 6.0 mg/m^2 (1.0 mg/kg; doses less than the recommended human daily dose of ONUREG) although treatment during the preimplantation period (on gestation days 1-3) had no adverse effect on the embryos at this dose.

Azacitidine caused an increase in fetal death and resorptions when administered as a single IP dose of 3 to 12 mg/m^2 on gestation days 9 or 10; average live animals per litter was reduced to 9% of control at the highest dose on gestation day 9. Multiple-statistically significant fetal anomalies were observed in rats following IP dosing of ≥ 1.8 mg/m^2 (0.3 mg/kg) on gestation day 1 to 8, or after a single IP dose of 3 to 12 mg/m^2 (0.5 to 2 mg/kg, respectively) on gestation day 9, 10, 11 or 12. Findings included: CNS anomalies (exencephaly/encephalocele), limb anomalies (micromelia, club foot, syndactyly, oligodactyly) and others (microphthalmia, micrognathia, gastroschisis, oedema, and rib abnormalities). Doses in these studies ranged from 1.0 to 6.5% of the recommended human daily dose of ONUREG® on a mg/m^2 basis.

Safety Pharmacology:

An *in vivo* safety pharmacology study in dogs receiving azacitidine at single intravenous doses of ≥ 2 mg/kg (≥ 40mg/m^2) reported increased heart rate, decreased blood pressure and increased QTc interval. The interpretations of this study are limited by concurrent confounding effects associated with toxicity including severe clinical signs (e.g., vomiting, flushed skin, decreased food consumption, and decreased spontaneous locomotor activity as well as fecal changes such as watery, mucous, or loose stool) in dogs. Additionally, large excursions in heart rate, decreases in potassium, and concurrent elevated autonomic nervous system tone as indicated by a spectrum of clinical signs and tachycardia limit the interpretation of the measurements. Results from *in vitro* tissue studies support the conclusion that azacitidine had no direct effects on vasodilatory parameters in the isolated rat aorta, no positive chronotropic effect was observed on the pacemaker activity of the guinea pig right atria, and there was no effect on heart rate and contractility in the isolated perfused guinea pig hearts; therefore, suggesting that blood pressure and heart rate changes in the cardiovascular dog study were also due to indirect effects of azacitidine-related toxicity.

In single-dose safety pharmacology studies in rats, azacitidine affected different central nervous system (CNS)-functional parameters including decreases in locomotor activity and muscle tone as well as altered several respiratory functional parameters including reductions in respiratory rate and tidal/minute volumes at an intravenous dose range of 10 to 40 mg/kg (60 to 240 mg/m^2).

READ THIS FOR SAFE AND EFFECTIVE USE OF YOUR MEDICINE

PATIENT MEDICATION INFORMATION

Pr ONUREG®
azacitidine tablets

Read this carefully before you start taking **ONUREG®** and each time you get a refill. This leaflet is a summary and will not tell you everything about this drug. Talk to your healthcare professional about your medical condition and treatment and ask if there is any new information about **ONUREG®.**

What is ONUREG® used for?

ONUREG® is for use in adults that have no more signs (remission) of a blood cancer called acute myeloid leukemia (AML). ONUREG® maintains remission of AML in patients:

- who may or may not have received an additional phase of chemotherapy (consolidation therapy), and
- who are not able to receive a stem cell transplant.

How does ONUREG® work?

ONUREG® helps prevent cancer cells from growing. Azacitidine disrupts the production of new genetic building blocks of cells. This helps kill cancer cells in leukemia, a type of blood cancer.

Talk to your healthcare professional if you have any questions about how ONUREG® works or why this drug has been prescribed for you.

What are the ingredients in ONUREG®?

Medicinal ingredients: azacitidine

Non-medicinal ingredients: black iron oxide, croscarmellose sodium, hypromellose, iron oxide red, iron oxide yellow, lactose monohydrate, polyethylene glycol/macrogol, magnesium stearate, mannitol, silicified microcrystalline cellulose, titanium dioxide, triacetin

ONUREG® comes in the following dosage forms:

Tablets: 200 mg or 300 mg

Do not use ONUREG® if:

- You are allergic to any ingredients in this drug or the container.
- You have advanced liver cancer.
- You have a blood cancer called myelodysplastic syndrome (MDS) unless you are participating in a controlled trial.

To help avoid side effects and ensure proper use, talk to your healthcare professional before you take ONUREG®. Talk about any health conditions or problems you may have, including if you:

- are pregnant, or you or your partner are planning on getting pregnant
- are breastfeeding or planning to breastfeed. You should not breastfeed while taking ONUREG®.

- Have a blood cancer called myelodysplastic syndromes (MDS).
- Have kidney or liver problems.
- Have heart problems.

Other warnings you should know about:

- ONUREG® is not switchable with, and should not be substituted with or for, azacitidine for injection.

Fertility:
- ONUREG® may harm fertility in males and females. If you wish to have or father children, talk to your healthcare professional about ways to keep your fertility before you start treatment

Female Patients: Important Warnings
- ONUREG® should not be used if you are pregnant. ONUREG® may cause harm to your unborn baby.
- If you can get pregnant, your healthcare professional will do a pregnancy test before starting ONUREG®.
- If you are pregnant or become pregnant while taking ONUREG®, tell your healthcare professional right away. They will explain the risks to you.
- You must use an effective birth control methods while taking ONUREG® and for at least 6 months after your last dose. Talk to your healthcare professional about birth control methods that may be right for you.

Breast-Feeding:
- Do not breastfeed during treatment with ONUREG® and for at least one week after the last dose. It is not known if ONUREG® passes into the mother's milk.

Male Patients: Important Warnings
- While taking ONUREG® and for at least 6 months after your last dose:
    - Your female partner should not get pregnant by you, and
    - You must use an effective birth control methods. Talk to your healthcare professional about birth control methods that may be right for you.

ONUREG® can cause serious side effects, including:
- **Stomach problems such as nausea, vomiting and diarrhea.** Your healthcare professional may give you drugs to treat or prevent these symptoms.
- **Blood problems:** ONUREG® can cause **neutropenia** (low levels of white blood cells, sometimes with a fever) and **thrombocytopenia** (low levels of platelets). Your healthcare professional may adjust how you take ONUREG® if you experience these side effects.

  For more information on these and other serious side effects, (their) symptoms and what to do about them, see the "Serious side effects and what to do about them" table. The table can be found later in this Patient Medication Information.

Driving and Using Machines: Before you do tasks which may require special attention, wait until you know how you respond to ONUREG®. If you feel tired, weak or have stomach problems, do not drive or use tools or machines.

Tell your healthcare professional about all the medicines you take, including any drugs, vitamins, minerals, natural supplements or alternative medicines.

The following may interact with ONUREG®:

No relevant interactions are known.

How to take ONUREG®:

- To prevent nausea and vomiting, your healthcare professional may recommend you take another drug before you take your dose of ONUREG®. Your healthcare professional will review the ongoing need for this additional drug.
- Take ONUREG® as your healthcare professional tells you.
- Take ONUREG® by mouth at about the same time each day.
- Swallow the tablets whole with water. Do not split, crush or chew the tablets.
- Take ONUREG® with or without food.
- Do not take more than the recommended dose prescribed by your healthcare professional.
- Do not change the ONUREG® dose or schedule unless your healthcare professional tells you to.

Recommended Dose:
Adults:

- Take 300 mg once daily. ONUREG® is taken for 14 days followed by a rest period of 14 days. This treatment cycle will be repeated every 28 days.
- Your healthcare professional will monitor your health. They may interrupt, reduce or stop your dose. This may occur based on your current health, if you take certain other medications or if you have certain side effects.

Overdose:

If you think you have taken too much ONUREG®, contact your healthcare professional, hospital emergency department or regional poison control centre immediately, even if there are no symptoms.

Missed Dose:
If a dose of ONUREG® is missed or not taken at the usual time:

- Take the missed dose as soon as you remember. Take the next dose at your regular time on the next day.
- Do not take two doses on the same day.
- If you vomit after taking a dose of ONUREG®, do not take another dose on that same day. Take your next dose at your regular time on the next day.

What are possible side effects from using ONUREG®?

These are not all the possible side effects you may feel when taking ONUREG®. If you experience any side effects not listed here, contact your healthcare professional.

Side effects may include:

- anxiety

- tiredness / weakness
- constipation
- stomach area pain
- excess gas
- pain in your joints, back, arms and/or legs
- decreased appetite
- dizziness

Your healthcare professional will do some tests before, during and after your treatment. These include blood tests to check your blood cell count. More frequent tests might be needed. They will tell you if your test results are abnormal and if you need treatment.

Serious side effects and what to do about them			
Symptom / effect	Talk to your healthcare professional		Stop taking drug and get immediate medical help
	Only if severe	In all cases	
VERY COMMON			
Neutropenia or Leukopenia (decreased white blood cells): infections, fatigue, fever, aches, pains and flu-like symptoms		√	
Thrombocytopenia (low blood platelets): bruising or bleeding for longer than usual if you hurt yourself, fatigue and weakness		√	
Anemia (decreased number of red blood cells): fatigue, loss of energy, irregular heartbeats, pale complexion, shortness of breath, weakness		√	
Nausea, vomiting, diarrhea		√	
COMMON			
Pneumonia (infection in the lungs): chest pain when you breath or cough, confusion, cough which may produce phlegm, fatigue, fever, sweating and shaking chills, nausea, vomiting or diarrhea, shortness of breath			√
Syncope (fainting)**:** a temporary loss of consciousness due to a sudden drop in blood pressure		√	
Upper respiratory tract infection (a cold or flu): runny or stuffy nose, sore throat, cough, sinus congestion, body		√	

aches, headache, sneezing, fever, generally feeling unwell			
Urinary tract infection (infection in urinary system including kidneys, ureters, bladder and urethra): Pain or burning sensation while urinating, frequent urination, blood in urine, pain in the pelvis, strong smelling urine, cloudy urine		√	

If you have a troublesome symptom or side effect that is not listed here or becomes bad enough to interfere with your daily activities, talk to your healthcare professional.

Reporting Side Effects

You can report any suspected side effects associated with the use of health products to Health Canada by:

- Visiting the Web page on Adverse Reaction Reporting (https://www.canada.ca/en/health-canada/services/drugs-health-products/medeffect-canada/adverse-reaction-reporting.html) for information on how to report online, by mail or by fax; or
- Calling toll-free at 1-866-234-2345.

NOTE: Contact your health professional if you need information about how to manage your side effects. The Canada Vigilance Program does not provide medical advice.

Storage:
Store blisters at 15°C to 30°C. Store in the original aluminum blisters.

Wash your hands and skin with soap and water right away after contact with ONUREG® tablets. If tablets come in contact with mucous membranes (soft, moist areas inside the openings of your body), flush the area with water right away.

Keep out of reach and sight of children.

Do not throw away any drugs via wastewater or household waste. Ask your pharmacist how to throw away drugs you no longer use.

If you want more information about ONUREG®:
- Talk to your healthcare professional
- Find the full product monograph that is prepared for healthcare professionals and includes this Patient Medication Information by visiting the Health Canada website (https://health-products.canada.ca/dpd-bdpp/index-eng.jsp); the manufacturer's website www.celgene.ca, or by calling 1-800-923-5436.

This leaflet was prepared by Celgene Inc., a Bristol-Myers Squibb company

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Last Revised: JAN-04-2021

Attachment A-5

EUROPEAN
COMMISSION

Brussels, 17.6.2021
C(2021) 4584 final

COMMISSION IMPLEMENTING DECISION

of 17.6.2021

granting marketing authorisation under Regulation (EC) No 726/2004 of the European Parliament and of the Council for "Onureg - azacitidine", a medicinal product for human use

(Text with EEA relevance)

(ONLY THE ENGLISH TEXT IS AUTHENTIC)

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of 17.6.2021

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(Text with EEA relevance)

(ONLY THE ENGLISH TEXT IS AUTHENTIC)

THE EUROPEAN COMMISSION,

Having regard to the Treaty on the Functioning of the European Union,

Having regard to Regulation (EC) No 726/2004 of the European Parliament and of the Council of 31 March 2004 laying down Community procedures for the authorisation and supervision of medicinal products for human and veterinary use and establishing a European Medicines Agency[1], and in particular Article 10(2) thereof,

Having regard to the application submitted by Bristol-Myers Squibb Pharma EEIG, on 21 May 2020, under Article 4(1) of Regulation (EC) No 726/2004,

Having regard to the opinion of the European Medicines Agency, formulated on 22 April 2021 by the Committee for Medicinal Products for Human Use,

Whereas:

(1) The medicinal product "Onureg - azacitidine" complies with the requirements set out in Directive 2001/83/EC of the European Parliament and of the Council of 6 November 2001 on the Community code relating to medicinal products for human use[2].

(2) It is therefore appropriate to authorise its placing on the market.

(3) The measures provided for in this Decision are in accordance with the opinion of the Standing Committee on Medicinal Products for Human Use,

HAS ADOPTED THIS DECISION:

Article 1

The marketing authorisation provided for in Article 3 of Regulation (EC) No 726/2004 is granted for the medicinal product "Onureg - azacitidine", the characteristics of which are summarised in Annex I to this Decision. "Onureg - azacitidine" shall be registered in the Union Register of Medicinal Products under number EU/1/21/1556.

[1] OJ L 136, 30.4.2004, p. 1.
[2] OJ L 311, 28.11.2001, p. 67.

Article 2

The marketing authorisation concerning the medicinal product referred to in Article 1 shall be subject to compliance with the conditions set out in Annex II and, in particular, with those relating to manufacture and importation, control and issue.

Article 3

The labelling and package leaflet concerning the medicinal product referred to in Article 1 shall comply with the conditions set out in Annex III.

Article 4

The period of validity of the authorisation shall be five years from the date of notification of this Decision.

Article 5

This Decision is addressed to Bristol-Myers Squibb Pharma EEIG, Plaza 254, Blanchardstown Corporate Park 2, D15 T867, Dublin 15, Ireland.

Done at Brussels, 17.6.2021

For the Commission

Sandra GALLINA
Director-General

CERTIFIED COPY
For the Secretary-General

Martine DEPREZ
Director
Decision-making & Collegiality
EUROPEAN COMMISSION

Attachment A-6

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Pharmaceutical Press

FASTtrack

Pharmaceutics – Drug Delivery and Targeting

Yvonne Perrie
Thomas Rades

fast track

Pharmaceutics - Drug Delivery and Targeting

Yvonne Perrie
Chair in Drug Delivery
Aston Pharmacy School
Aston University, Birmingham, UK

Thomas Rades
Chair in Pharmaceutical Sciences
The New Zealand National School of Pharmacy
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Preface

The best drugs can't exert their beneficial effects if they are not reaching their target site in the body at an appropriate concentration and for a sufficient length of time. It is clear that if a tablet is still in the package it is of no benefit for the patient. Less obvious is that this may also be the case after the tablet has been swallowed.

In the stomach the tablet might not disintegrate and the drug might not be released from the dosage form. Or the drug simply has too low aqueous solubility in the gastrointestinal fluids. If the drug is not in solution it will (generally) not be able to overcome the epithelial membranes of the gastrointestinal tract and it cannot enter into the body and reach the target site of its activity. *How can we improve the solubility of poorly water-soluble drugs?*

Some drugs chemically or enzymatically degrade in the stomach. Others lead to gastric irritation. *How can these drugs still be administered via the oral route?*

In other cases the drug may dissolve very fast and is absorbed very quickly from the gastrointestinal tract. Whilst this seems a good thing (and indeed it often is), it can lead to high plasma concentration peaks of the drug followed by fast elimination of the drug, and consequently a short duration of action. So the patient has to take the drug very frequently and there will be strong fluctuations in the plasma concentration of the drug over time. *Can we formulate a dosage form to improve this situation?*

Some drugs cannot be delivered by the oral route as they are metabolised in the liver, before reaching the systemic circulation. *What can be done?*

Again other drugs may have a strong side-effect profile, which may prohibit efficient treatment. *Can we improve this situation, and direct the drug to its target site, rather than having it distributed evenly throughout the body?*

This book tries to answer these and other questions in a concise and structured way. We will systematically review important concepts and facts relating to the delivery and targeting of drugs. The emphasis of this book has not been placed on the anatomical routes of delivery, but instead on the principles of drug release and targeting, namely: immediate release, delayed release, sustained release, controlled release and targeted release. Because of its overwhelming importance in drug delivery today, we have placed some focus on oral delivery (for immediate release, delayed release, sustained release), but other routes of delivery will also be covered where appropriate (for controlled and targeted release). Relevant examples of delivery systems will be given throughout the book with a focus on delivery systems that have actually reached clinical reality.

This book aims to highlight differences and principles in a concise way (including some mind maps at the end of the book), and so we have to apologise to the experts in the field if we have drawn boundaries for clarity where a grey zone is more appropriate. In the words of Picasso, however, we feel that students should know the boundaries before they can (and should) cross them.

Yvonne Perrie
Thomas Rades
July 2009

Chapter 1
Controlling drug delivery

Overview

In this chapter we will:

- differentiate drug delivery systems according to their physical state
- differentiate drug delivery systems according to their route of administration
- differentiate drug delivery systems according to their type of drug release
- discuss drug transport across epithelial barriers.

Introduction

Pharmacotherapy can be defined as the treatment and prevention of illness and disease by means of drugs of chemical or biological origin. It ranks among the most important methods of medical treatment, together with surgery, physical treatment, radiation and psychotherapy. There are many success stories concerning the use of drugs and vaccines in the treatment, prevention and in some cases even eradication of diseases (e.g. smallpox, which is currently the only human infectious disease completely eradicated). Although it is almost impossible to estimate the exact extent of the impact of pharmacotherapy on human health, there can be no doubt that pharmacotherapy, together with improved sanitation, better diet and better housing, has improved people's health, life expectancy and quality of life.

Unprecedented developments in genomics and molecular biology today offer a plethora of new drug targets. The use of modern chemical synthetic methods (such as combinatorial chemistry) enables the syntheses of a large number of new drug candidates in shorter times than ever before. At the same time, a better understanding of the immune system and rapid progress in molecular biology, cell biology and microbiology allow the development of modern vaccines against old and new challenges.

Key Points

- Continued developments in chemistry, molecular biology and genomics support the discovery and developments of new drugs and new drug targets.
- The drug delivery system employed can control the pharmacological action of a drug, influencing its pharmacokinetic and subsequent therapeutic profile.

Tip

Combinatorial chemistry is a way to build a variety of structurally related drug compounds rapidly and systematically. These are assembled from a range of molecular entities which are put together in different combinations. A 'library' of compounds of tens of thousands of different molecules is then screened to identify compounds that bind to therapeutic targets.

However, for all these exciting new drug and vaccine candidates, it is necessary to develop suitable dosage forms or drug delivery systems to allow the effective, safe and reliable application of these bioactive compounds to the patient. It is important to realise that the active ingredient (regardless of whether this is a small-molecular-weight 'classical' drug or a modern 'biopharmaceutical' drug like a therapeutic peptide, protein or antigen) is just one part of the medicine administered to the patient and it is the formulation of the drug into a dosage form or drug delivery system that translates drug discovery and pharmacological research into clinical practice.

Tip

Usually the drug concentration in the body is determined in the plasma. This is done as the plasma is comparatively easy to access and drug concentrations can be reliably measured using techniques such as high-performance liquid chromatography (HPLC). However, the desired site of action for most drugs is not the plasma and in principle it would be better to determine the drug concentration at the site of action of the drug.

Indeed the drug delivery system employed plays a vital role in controlling the pharmacological effect of the drug as it can influence the pharmacokinetic profile of the drug, the rate of drug release, the site and duration of drug action and subsequently the side-effect profile. An optimal drug delivery system ensures that the active drug is available at the site of action for the correct time and duration. The drug concentration at the appropriate site should be above the minimal effective concentration (MEC) and below the minimal toxic concentration (MTC). This concentration interval is known as the therapeutic range and the concept is illustrated in Figure 1.1, showing the drug plasma levels after oral administration of a drug from an immediate-release dosage form.

Achieving the desired concentration of a drug is dependent on the frequency of dosing, the drug clearance rates, the route of administration

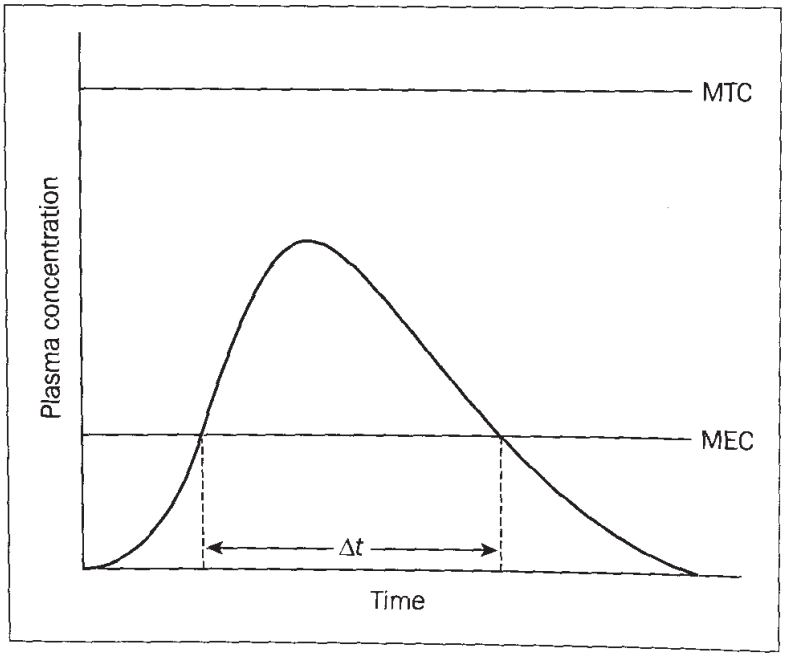


Figure 1.1 Drug plasma levels after oral administration of a drug from an immediate-release dosage from. The therapeutic range is the concentration interval between the minimal effective concentration (MEC) and the minimal toxic concentration (MTC). Δt is the time interval the drug is in the therapeutic range.

and the drug delivery system employed. Within this book the terms drug delivery system, dosage form and medicine are used interchangeably. However the term dosage form is often used to refer to the physical appearance of the medicine whereas the term delivery system is often used to refer to the way the medicine releases the drug and delivers it to the body or more specifically to the target organ, tissue, cell or even cellular organelle.

Differentiating delivery systems according to their physical state

For dosage forms it is common to differentiate the various types by classifying them according to their physical state into gaseous (e.g. anaesthetics), liquid (e.g. solutions, emulsions, suspensions), semisolid (e.g. creams, ointments, gels and pastes) and solid dosage forms (e.g. powders, granules, tablets and capsules). Most dosage forms contain several phases.

Sometimes the phases of a dosage form are of the same state, for example for an emulsion which contains two liquid phases (oil and water). Whilst both phases are liquid, they differ in their physical properties, for example density and electrical conductivity, and are separated from each other by an interface. However, more often the dosage form contains phases of different states. For example, a suspension contains a liquid and a solid phase. Therefore classification into gaseous, liquid, semisolid or solid dosage forms may sometimes appear somewhat arbitrary. Finally, in these multiphase dosage forms usually one or more phases are dispersed, whilst other phases are continuous. In a suspension the solid phase is dispersed and the liquid phase is continuous, and in an oil-in-water emulsion the oil phase is dispersed and the water phase is continuous. In some dosage forms the determination of the type and number of phases is not as straightforward. For example, the phases of creams can be difficult to determine, with the presence of a dispersed water (or oil) phase in addition to several continuous phases (oil, water and surfactant phases). For liposomal dispersions, the state of the phospholipids

Key Points

- Dosage forms can be classified according to their physical state.
- Most dosage forms contain several phases.
- Systems containing a dispersed phase will give rise to physical instability issues.
- All systems move to a state of minimum free energy.

Tip

A phase is a volume element of a system (here the dosage form), separated from other volume elements of the system by a phase boundary (interface to another phase). The physical properties within the phase do not vary, which means that the phase is physically homogeneous. From the requirement of homogeneity within a phase it follows that the number of molecules within the phase is large compared to the number of molecules forming the interface between the phases and surrounding other phases.

Tip

To understand dosage forms from a physical perspective, try to identify the number of phases in a dosage form, their state and if they are dispersed or continuous.

Tips

Here are some examples of how dosage forms in their simplest terms can be differentiated according to the state and dispersion of their phases:

- A drug solution is a one-phase system as the dissolved drug does not fulfil the requirements for a phase. In a solution the molecularly dispersed drug will not separate out to form larger particles if the concentration of the drug is not changed (e.g. by evaporation of the solvent) and the environmental conditions (e.g. temperature) are constant.
- A suspension is a two-phase system containing a continuous liquid phase and a dispersed solid phase.
- An emulsion is a two-phase system containing two liquid phases, one dispersed and one continuous.
- Ointments are generally two-phase or multiphase gels, with at least two continuous phases (usually a crystalline or liquid crystalline surfactant phase and a lipid phase).
- Creams additionally contain a water phase which may be dispersed (water-in-oil cream) or continuous (oil-in-water cream).
- Tablets are essentially compressed powers, and might thus be classified as containing a solid and gaseous continuous phase. Of course a tablet contains several solid phases, as drug particles are usually present together with other solid phases (e.g. filler, binder, disintegrant, glidant and lubricant particles).

used to form the liposomes will determine if a liposomal dispersion is a suspension (if the lipids are in a crystalline state) or an emulsion (if the lipids are in a fluid, liquid crystalline state).

It is important to note that the presence of a dispersed phase will lead to physical instability in the system. For example, in an oil-in-water emulsion, the dispersed oil droplets have a larger interfacial area to the water than if the droplets had coalesced into one large continuous phase. This increased interfacial area leads to an increased interfacial free energy, according to the relationship:

$$G_i = A\gamma$$

where G_i is the interfacial free energy of the system, A is the interfacial area between the dispersed phase (here the oil droplets) and the continuous phase (here the water phase) and γ is the interfacial tension between the two phases. The interfacial free energy of the system (here the emulsion) can be minimised by coalescence of the droplets into larger droplets and finally into one continuous oil phase, as this maximally reduces the total interfacial area. This is of course undesirable from a formulation viewpoint. Coalescence of droplets in an emulsion is a pharmaceutical instability, but from a thermodynamic viewpoint the system has been stabilised, as the interfacial free energy has been reduced. In practical terms an emulsion is pharmaceutically stabilised by adding emulsifiers to the systems, that either lower the interfacial tension (note: if γ gets smaller, G_i will get smaller), or that act as a physical barrier against coalescence. In either case, increasing the interfacial area will still increase the surface free energy.

Differentiating delivery systems according to their route of administration

Another way of differentiating dosage forms is according to their site or route of administration. Drugs can be administered directly into the

body, though injection or infusion. This form of drug administration is termed parenteral drug delivery. Depending on the site of administration into the body one can differentiate between intravenous, intramuscular, subcutaneous, intradermal and intraperitoneal administration. Usually aqueous solutions are used for intravenous delivery, but it is also possible that the dosage form contains a dispersed phase (solid or liquid), provided the dispersed particles are small enough (e.g. smaller than 100–150 nm) to avoid embolism. For other routes of parenteral administration the delivery systems can be aqueous or oily or even solid (the latter dosage forms are termed implants).

Drugs can also be administered on to the skin to enter into the body. Mostly semisolid dosage forms are used for this, including creams, ointments, gels and pastes. However, liquid dosage forms, such as emulsions, or solid dosage forms, such as transdermal controlled drug delivery systems (patches), can also be used. These will be discussed in more depth in Chapter 6. It has to be taken into account, though, that one of the main functions of the skin as an organ is to prevent particles or compounds entering the body, rather than allowing them to be absorbed into the body. The stratum corneum of the skin forms a formidable barrier against uptake and thus transdermal delivery is difficult to achieve. Penetration enhancers often have to be added to the delivery system to improve delivery into or through the skin. In transdermal controlled drug delivery systems ideally the dosage form controls the uptake into the skin (rather than the uptake being controlled by the stratum corneum).

The most important route of drug administration into the body is through mucosal membranes. Mucosal membranes are much less of a barrier to uptake than the skin and some mucosal membranes (such as the ones in the small intestine) are indeed

KeyPoints

- The various routes of administration of a drug into the body can be generally classified into:
  - direct entry into the body
  - entry into the body by overcoming the skin
  - entry into the body by overcoming mucosal membranes.
- The oral route is often the most convenient route for drug delivery; however, drugs delivered via this route can be metabolised by the hepatic first-pass effect.

Tips

Parenteral drug delivery

Subcutaneous injection
The needle is inserted into the fatty tissue just under the skin. Volumes should be less than 2.5 ml per injection site. Insulin is commonly administered via this route.

Intramuscular injection
Injection into the muscle is preferred to the subcutaneous route if larger volumes (typically up to 5 ml in appropriate sites) have to be given.

Intravenous injection
A needle is inserted directly into a vein. This is the easiest way to give a precise dose rapidly. Small volumes can be given as a single dose whereas larger volumes can be given by infusion.

Intradermal injection
Injection is given into the skin. This form of parenteral administration is used in allergy skin testing.

Intraperitoneal injection
Injection through the peritoneum (the thin, transparent membrane that lines the walls of the abdomen).

Tips

The US Food and Drug Administration (FDA) has compiled an extensive list of different dosage forms and different routes of administration. These lists can be found on the FDA Center for Drug Research websites:

- http://www.fda.gov/cder/dsm/DRG/drg00201.htm
- http://www.fda.gov/cder/dsm/DRG/drg00301.htm

specialised sites for absorption. There are many mucosal membranes that can be used for drug administration. Of the highest importance are the mucosal membranes of the gastrointestinal tract, allowing oral drug delivery. The suitability and convenience of this route of delivery make oral dosage forms the most common of all drug delivery systems. Also the buccal, sublingual, rectal and vaginal mucosa and indeed the lung and nasal mucosal membranes can act as absorption sites. For all of these mucosal membranes dosage forms have been developed, such as buccal and sublingual tablets, suppositories, vaginal rings, inhalers and nasal sprays, to name a few.

If drug delivery systems are designed to give a local drug effect and not systemic activity, they can be described as topical delivery systems. This is the case for many dermal dosage forms.

Oral drug delivery

As stated above, the oral route is the most popular route to administer drugs. However, some factors should be considered when looking to administer drugs via this route. In particular the transit time in the gastrointestinal tract may vary considerably:

- between patients and within the same patient, with the gastric residence time being the most variable
- with the state of the dosage form (liquid dosage forms are emptied out of the stomach faster than solid dosage forms)
- with the fasted or fed state of the patient.

The pH conditions in the gastrointestinal tract also vary considerably, from a low pH in the stomach (1.5–2 in the fasted state to around 5 in the fed state) to a higher pH in the small and large intestine. The pH in the small intestine varies from 4 to 7, with an average value of approximately 6.5. This may affect stability and will influence the degree of ionisation of ionisable drugs which in turn will influence their absorption (unionised forms of drugs are usually taken up better than ionised forms of the same drug) and solubility (unionised forms are usually less soluble than ionised forms of the same drug).

First-pass metabolism

Importantly, drugs that are taken up into the body through the gastrointestinal mucosa will be transported to the liver via the portal vein before going into general circulation. As the liver is the main metabolic organ of the body, if the drug is susceptible to metabolic degradation in the liver, this may considerably reduce the activity of

the drug. This phenomenon is known as the hepatic first-pass effect. The rectal route may also show varying degrees of the first-pass effect, while for other routes of administration (intravenous, vaginal, nasal, buccal and sublingual) the drug is distributed in the body before reaching the liver, and therefore for certain drugs these may be the preferred route of administration. However, whilst the liver is the main metabolic organ of the body, metabolism may also take place in the gastrointestinal lumen and indeed in the mucosal membranes.

Tip

After oral administration first-pass metabolism may occur in the liver and the gut. For example, glyceryl trinitrate is predominantly metabolised in the liver and is therefore often formulated for sublingual delivery. In contrast, benzylpenicillin and insulin are primarily metabolised in the gut lumen while orlistat is metabolised within the gastrointestinal mucosal membrane.

Differentiating drug delivery systems according to their mechanism of drug release

Another systematic that can be used to differentiate drug delivery systems is according to the way the drug is released. Broadly, one can differentiate as follows:

- Immediate release – drug is released immediately after administration.
- Modified release – drug release only occurs some time after the administration or for a prolonged period of time or to a specific target in the body. Modified-release systems can be further classified as:
  - Delayed release: drug is released only at some point after the initial administration.
  - Extended release: prolongs the release to reduce dosing frequency.

These terms are also used by the pharmacopoeias and the FDA. Whilst immediate-release dosage forms are designed to give a fast onset of drug action, modifications in drug release are often desirable to increase the stability, safety and efficacy of the drug, to improve the therapeutic outcome of the drug treatment and/or to increase patient compliance and convenience of administration.

KeyPoints

- Dosage forms can control the rate of release of a drug and/or the location of release.
- They can be classified into immediate-release and modified-release dosage forms.
- The modified-release systems can be further divided into delayed-, extended- and targeted-release systems.
- Extended-release systems can be further divided into sustained- and controlled-release systems.
- Modifications in drug release profiles can be used to improve the stability, safety, efficacy and therapeutic profile of a drug.

Tips

The various forms of release as defined by the FDA

Immediate release
Allows the drug to dissolve in the gastrointestinal contents, with no intention of delaying or prolonging the dissolution or absorption of the drug.

Modified release
Dosage forms whose drug release characteristics of time course and/or location are chosen to accomplish therapeutic or convenience objectives not offered by conventional dosage forms such as a solution or an immediate-release dosage form. Modified-release solid oral dosage forms include both delayed- and extended-release drug products.

Delayed release
Release of a drug (or drugs) at a time other than immediately following oral administration.

Extended release
Extended-release products are formulated to make the drug available over an extended period after ingestion. This allows a reduction in dosing frequency compared to a drug presented as a conventional dosage form (e.g. as a solution or an immediate-release dosage form).

No definition for controlled release or targeted release is provided by the FDA or pharmacopoeias.

KeyPoints

- Immediate-release delivery systems give a fast onset of action.
- For a therapeutic action the drug should be in solution, therefore disintegration of the dosage form and dissolution of the drug may have to occur first depending on the dosage form.
- Immediate-release systems usually release the drug in a single action following a first-order kinetics profile.
- The time of action of the drug is limited to the time that the concentration of the drug is above the MEC.

Immediate release

Many dosage forms are designed to release the drug immediately or at least as quickly as possible after administration. This is useful if a fast onset of action is required for therapeutic reasons. For example, a tablet containing a painkiller should disintegrate quickly in the gastrointestinal tract to allow a fast uptake into the body.

The onset of action is very fast for intravenous injections and infusions and the pharmacological effect may be seen in a matter of seconds after administration. The reasons for this are twofold:

1. The drug is already in solution, so strictly speaking the drug does not have to be released from the dosage form at all.
2. The drug is directly administered into the body, so no time is lost due to drug permeation through the skin or mucosal membranes, before the target organs can be reached.

In oral solutions the drug is also already released and the solution will simply mix with the gastrointestinal fluids. However, powders and granules need to dissolve first before the drug is released by dissolution. For tablets it is initially necessary that the tablet disintegrates (if it is formed from compressed granules this will initially happen to the level of the granules, from which further disintegration into powder particles and finally drug dissolution occurs). For capsules to release their drug content it is necessary for the capsule shell material (for example, gelatin or hydroxypropylmethylcellulose (HPMC)) first to disintegrate. Thereafter the drug can either dissolve from the usually solid powders or granules in the case of hard gelatin or HPMC capsules or it can be dispersed from the usually liquid, lipophilic content of a soft gelatin capsule. These types of immediate-release dosage forms have an onset of action in the order of minutes to hours.

Immediate-release dosage forms usually release (dissolve or disperse) the drug in a single

action following a first-order kinetics profile. This means the drug is released initially very quickly and then passes through the mucosal membrane into the body, reaching the highest plasma level (termed C_{max}) in a comparatively short time (termed t_{max}). Uptake through the mucosal membranes may be due to passive diffusion or by receptor-mediated active transport mechanisms (see section on modified release). Once taken up into the body the drug is distributed throughout the body and elimination of the drug by metabolism and excretion occurs. The elimination process also usually follows first-order kinetics. Therefore the plasma levels measured over time after administration of an immediate-release dosage form (the plasma concentration time curve) basically are the sum of a first-order absorption and a first-order elimination process. The resulting function is known as the Bateman function. Figure 1.2 shows an idealised plasma concentration versus time profile of an immediate-release oral dosage form.

Tips

First-order kinetics
The rate of the process is proportional to the concentration of one of the reactants, in our case the drug.

Bateman function
This function was initially used to describe the concentration of a radioactive material *B* that stems from a first-order decay of another radioactive material *A* and that in its own right further decays to another material *C*. If both decay processes ($A \rightarrow B$ and $B \rightarrow C$) follow first-order kinetics, exactly the same function results as for the plasma concentration time curve of a drug from an immediate-release oral dosage form. The $A \rightarrow B$ decay is equivalent to the absorption process and the $B \rightarrow C$ process is equivalent to the elimination process.

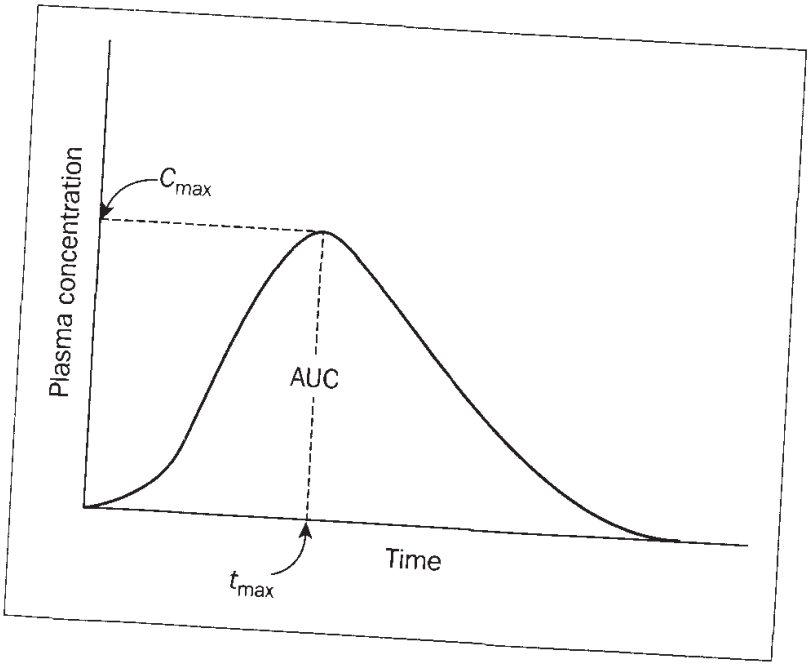


Figure 1.2 Idealised plasma concentration versus time profile of an immediate-release oral dosage form. The highest drug plasma concentration is termed C_{max}. The time at which C_{max} is reached is termed t_{max}. The area under the plasma concentration versus time profile is termed AUC and reflects the total amount of drug absorbed.

An important consideration for immediate-release dosage forms is that the time of action of the drug is limited to the time that the concentration of the drug is above the MEC. If the drug has a short biological half-life, this time interval may be short, requiring frequent

dosing and potentially leading to low patient compliance and suboptimal therapeutic outcome.

The biological half-life of a drug is defined as the time required to reduce the plasma concentration by 50% by metabolism or excretion. Many studies show that a large proportion of patients do not take drugs as directed (for example three times a day), especially if the disease is (at least initially) not accompanied by strong symptoms, for example in the treatment of high blood pressure or glaucoma. To reduce the frequency of drug administration it is often not possible simply to increase the dose of an immediate-release dosage form as the peak plasma concentrations may be too high and lead to unacceptable side-effects. Therefore the drug concentration within the plasma should be above the MEC and below the MTC, i.e. within the therapeutic range (Figure 1.1).

KeyPoints

- Modified-release systems are designed to influence the release profile of a drug from its delivery system.
- Oral delayed-release systems can delay release until specific regions of the gastrointestinal tract are reached.
- Extended release of a drug can be achieved using sustained- or controlled-release drug delivery systems.
- Controlled-release systems aim to control the plasma concentration of the drug after administration by various possible routes.

Tip

Immediate-release oral delivery systems can also have polymer coatings. In this case the polymer may be used to mask an unpleasant taste or odour, to facilitate swallowing of the drug or to improve identification of the medicine. These coats dissolve quickly in the stomach and do not delay the release of the drug.

Modified release

Dosage forms can be designed to modify the release of the drug over a given time or after the dosage form reaches the required location.

Delayed release

Delayed-release dosage forms can be defined as systems which are formulated to release the active ingredient at a time other than immediately after administration. Delayed release from oral dosage forms can control where the drug is released, e.g. when the dosage form reaches the small intestine (enteric-coated dosage forms) or the colon (colon-specific dosage forms).

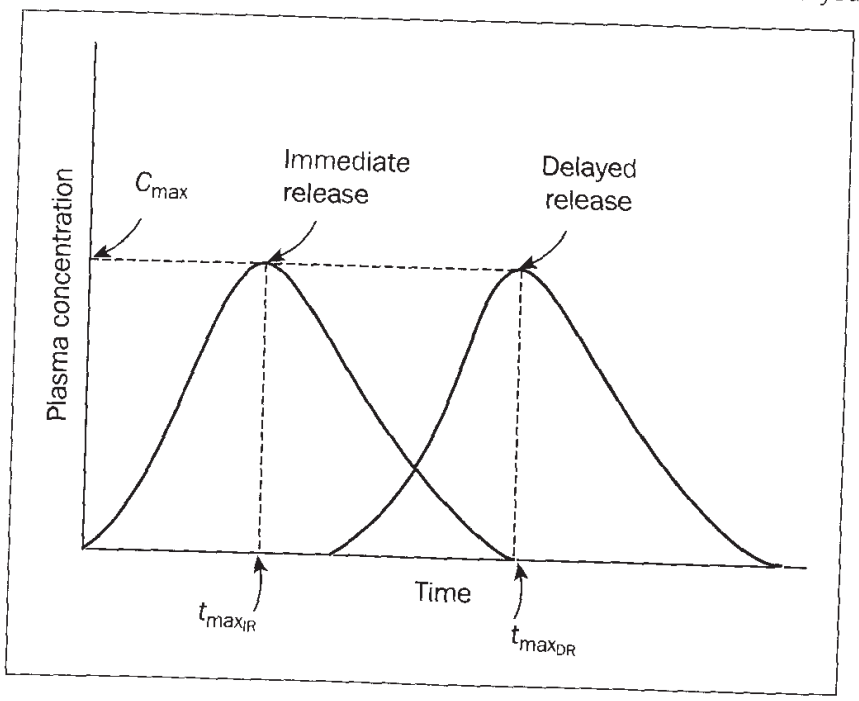
Delayed-release systems can be used to protect the drug from degradation in the low pH environment of the stomach or to protect the stomach from irritation by the drug. In these cases drug release should be delayed until the dosage form has reached the small intestine. Often polymers are used to achieve this aim. The dosage form (for example, a tablet or the granules before tableting) can be coated with a suitable polymer. The polymer dissolves as a function of pH, so when the dosage forms travel from the low-pH environment of the stomach to the higher-pH environment of the small intestine, the polymer coat dissolves and the drug can be released. Once this occurs, the release is again immediate and the resulting plasma

concentration versus time curve is similar to the one for immediate-release dosage forms.

The development of colon-specific drugs and dosage forms may be advantageous for the treatment of local and systemic diseases, including colorectal cancer and Crohn's disease. Especially for peptide and protein drugs, this form of release may also be advantageous for systemic administration given the more favourable pH conditions in the colon compared to the stomach and the generally lower enzymatic activity compared to the small intestine.

Figure 1.3 shows an idealised plasma concentration versus time profile of a delayed-release oral dosage form. T_{max} (but not C_{max}) is strongly dependent on the gastric emptying times which, as stated above, may be quite variable.

Figure 1.3 Idealised plasma concentration versus time profile of a delayed-release oral dosage form compared to an immediate-release dosage form. T_{maxIR} is the time for maximum plasma concentration of the drug released from an immediate-release dosage form and T_{maxDR} is the time for maximum plasma concentration of the drug released from a delayed-release dosage form.



Extended release

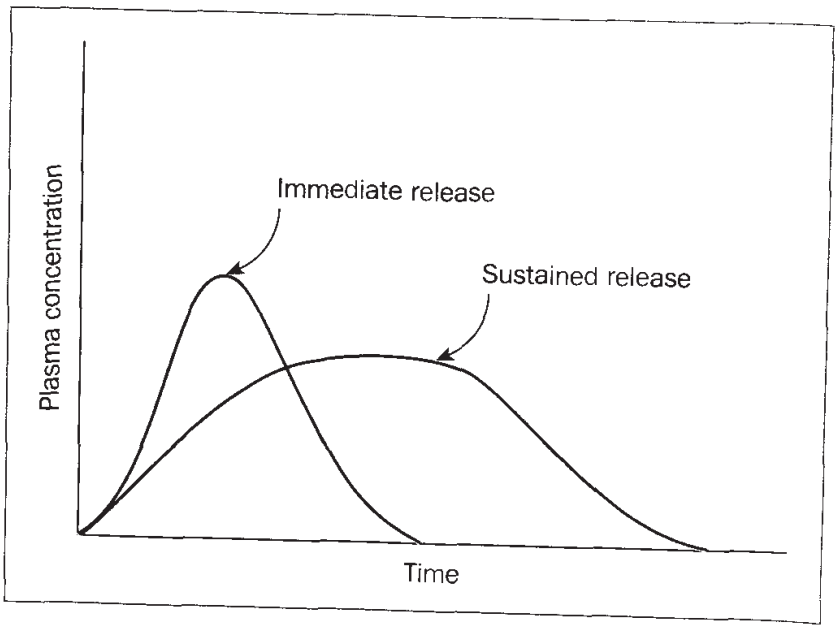
Extended-release systems allow for the drug to be released over prolonged time periods. By extending the release profile of a drug, the frequency of dosing can be reduced. For immediate-release dosage forms the time interval the plasma concentration is in the therapeutic range of the drug can be quite short. Therefore frequent dosing, with its associated compliance problems, is required. This is especially an issue in chronic diseases when patients need to take the medicine for prolonged periods of time, often for the rest of their life. Extended release can be achieved using sustained- or controlled-release dosage forms.

Sustained release

These systems maintain the rate of drug release over a sustained period (Figure 1.4). For example, if the release of the drug from the dosage form is sustained such that the release takes place throughout the entire gastrointestinal tract, one could reduce C_{max} and prolong the time interval of drug concentration in the therapeutic range. This in turn may reduce the frequency of dosing, for example from three times a day to once a day. Sustained-release dosage forms achieve this mostly by the use of suitable polymers, which are used either to coat granules or tablets (reservoir systems) or to form a matrix in which the drug is dissolved or dispersed (matrix systems). The release kinetics of the drug from these systems may differ:

- Reservoir systems often follow a zero-order kinetics (linear release as a function of time).
- Matrix systems often follow a linear release as a function of the square root of time.

Figure 1.4 Idealised plasma concentration versus time profile of a sustained-release oral dosage form compared to an immediate-release dosage form.



Controlled-release

Controlled-release systems also offer a sustained-release profile but, in contrast to sustained-release forms, controlled-release systems are designed to lead to predictably constant plasma concentrations, independently of the biological environment of the application site. This means that they are actually controlling the drug concentration in the body, not just the release of the drug from the dosage form, as is the case in a sustained-release system. Another difference between sustained- and controlled-release dosage forms is that the former are basically restricted

to oral dosage forms whilst controlled-release systems are used in a variety of administration routes, including transdermal, oral and vaginal administration.

Controlled release of drugs from a dosage form may be achieved by the use of so-called therapeutic systems. These are drug delivery systems in which the drug is released in a predetermined pattern over a fixed period of time. The release kinetics is usually zero-order. In contrast to sustained-release systems, the dose in the therapeutic systems is of less importance than the release rate from the therapeutic system. Ideally the release rate from the dosage form should be the rate-determining step for the absorption of the drug and in fact for the drug concentration in the plasma and target site. However, controlled-release systems are not necessarily target-specific, which means that they do not 'exclusively' deliver the drug to the target organ. This may be achieved by so-called targeted delivery systems which aim to exploit the characteristics of the drug carrier and the drug target to control the biodistribution of the drug. Figure 1.5 shows an idealised plasma concentration versus time profile of a controlled-release dosage form.

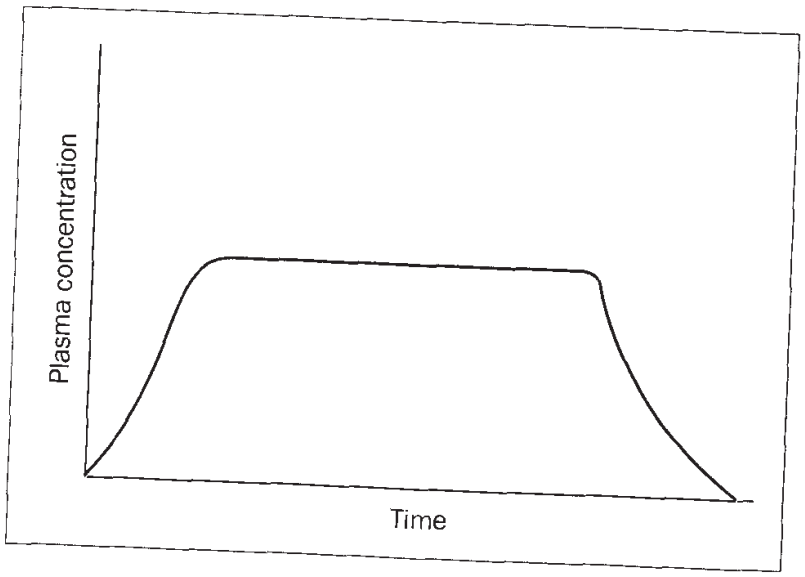


Figure 1.5 Idealised plasma concentration versus time profile of a controlled-release dosage form.

Optimum release profile

From immediate release and delayed release to sustained release and controlled release, we have seen that the resulting plasma concentration versus time curves have become increasingly flatter, prolonging the time the drug is in the therapeutic range after a single administration of the dosage form. This has led to the popular slogan: 'The flatter the better'. However, for some diseases it is advantageous to have varying release of the drug depending on the needs of the patient or circadian rhythms in the body. For example, insulin is needed in higher concentration after a meal and blood pressure has been found to be higher in the morning and

afternoon and drops off during the night. Patients with rheumatoid arthritis suffer from pain more strongly in the morning than in the night, whilst the situation is reversed for patients with osteoarthritis. It has also long been known that cortisol levels are higher in the morning and decline throughout the day. This has led to research into so-called feedback-regulated drug delivery systems in which the drug concentration (ideally at the drug target site) is measured through a sensor and, depending on the ideal drug concentration, release is either increased or slowed down. It is also possible that instead of the actual drug concentration a therapeutic effect is measured that then acts as a feedback for the drug release. These systems, however, have not yet entered the market.

KeyPoints

- Controlling the release rate of a drug does not ensure that the drug reaches the target site or is retained there.
- Passively targeted drug delivery systems can utilise the natural distribution mechanisms within the body.
- Active targeting of delivery systems uses targeting groups such as antibodies and ligands to direct the system to the appropriate target.

Targeted-release dosage forms

Whilst controlling the rate of release of a drug from its delivery system can control plasma drug concentration levels, once released there is often little control over the distribution of the drug in the body. Very few drugs bind exclusively to the desired therapeutic target and this can give rise to reduced efficacy and increased toxicity.

Drug targeting aims to control the distribution of a drug within the body such that the majority of the dose selectively interacts with the target tissue at a cellular or subcellular level. By doing so, it is possible to enhance the activity and specificity of the drug and to reduce its toxicity and side-effects. Drug targeting can be achieved by designing systems that passively target sites by exploiting the natural conditions of the target organ or tissue to direct the drug to the target site. Alternatively drugs and certain delivery systems can be actively targeted using targeting groups such as antibodies to bind to specific receptors on cells.

The differentiation of dosage forms according to drug release places the emphasis on the delivery of the drug and will be followed in this book.

KeyPoints

- The epithelial lining presents a barrier to drug absorption.
- Epithelia are classified based on their shape, number of cells that form the epithelial barrier and their specialisation.
- Mucus secreted from goblet cells presents an additional barrier to drug absorption.

Drug absorption

By using the various drug delivery strategies outlined above, it is possible to influence the distribution of a delivery system and the release of a drug from its delivery system. However, we must also consider the process of drug absorption after the drug has been released. The absorption of drugs is dependent on the site of absorption and the nature of the drug. Nearly all internal and external body surfaces,

and hence possible drug absorption routes, are lined with epithelial tissue. For example, drugs administered orally must cross the epithelium of the gastrointestinal tract before they can enter the systemic circulation.

Barriers to drug absorption

Epithelia are tissues composed of one or more layers of cells. These layers are supported by a basement membrane which lies on top of the supporting connective tissue. The function of epithelial cells includes absorption, secretion and protection and is dependent on their location within the body. The epithelia are classified by their:

1. *Shape*

a. Squamous – these cells have a flat (squashed) shape.

b. Columnar – these are narrow, tall cells.

c. Cuboidal – these cells have a cubic shape, intermediate between squamous and columnar.

2. *Stratification (number of cell layers)*

a. Simple – single layer of cells, termed epithelium.

b. Stratified – multiple layers.

3. *Specialisation – some epithelia will have a specialised function*

a. Keratinised cells contain keratin protein to improve the strength of the barrier.

b. Ciliated cells have apical membrane extensions that can increase the overall absorption area and rhythmically beat to move mucus.

Tips

Examples of epithelia include:

- Blood vessels: this epithelium lines the circulatory system and consists of a single layer of squamous cells.
- Oral: this epithelium consists of a single layer of columnar cells and lines the stomach and intestine. Cells of the small intestine have villi and microvilli to increase their surface area.
- Buccal: this epithelium consists of stratified squamous cells that may be keratinised.

Mucus

Many of the epithelial linings considered as absorption sites have a mucus layer coating. Mucus is synthesised and secreted by goblet cells which are a specialised type of columnar epithelial cells. Mucus is viscous in nature and is composed of highly glycosylated peptides known as mucins and inorganic salts in water. The main role of mucus is to protect and lubricate the epithelial lining. In the respiratory tract it supports mucociliary clearance, by trapping substances and removing them through the mucociliary escalator. In the gastrointestinal tract, mucus both protects the stomach from the acidic conditions therein and helps lubricate the passage of food.

However, in terms of drug delivery, mucus serves as a physical barrier to absorption. A substance must first diffuse across the mucus barrier before it can reach the epithelia and be absorbed. Therefore the viscosity and thickness of the mucus layer and any interactions the drug and/or delivery system may have with the mucus must be considered.

KeyPoints

- Drugs can cross epithelia by transcellular and paracellular mechanisms.
- The paracellular mechanism involves passive diffusion between cells.
- Transcellular diffusion involves movement through cells and may require energy.
- The route of transport is dependent on the physicochemical nature of the drug.

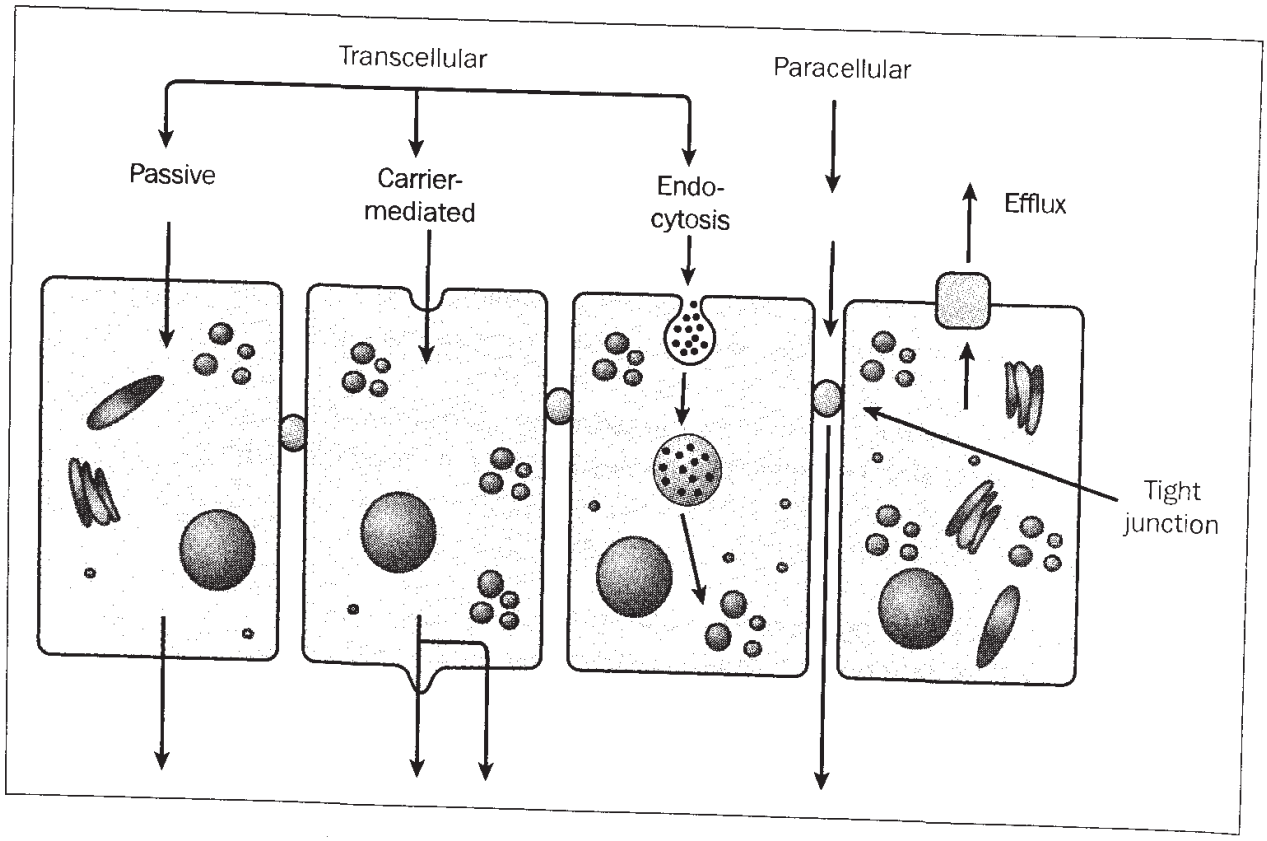
Mechanisms of drug absorption

The combination of the epithelial membranes and (where present) the mucus restricts the absorption of substances, including drugs. However there are mechanisms of absorption across the epithelial cells which involve:

- transcellular transport through cells
- paracellular transport between cells.

These mechanisms are summarised in Figure 1.6.

Figure 1.6 Transport processes across epithelial barriers.



Transcellular route

Passive diffusion

This involves the diffusion of drugs across the lipid bilayer of the cell membrane and is driven by a concentration gradient with drugs moving

from high to low concentration. The rate of diffusion is governed by Fick's law. Low-molecular-weight drugs are absorbed by passive diffusion and factors controlling the rate of diffusion include:

- drug concentration
- partition coefficient of the drug
- area of absorptive tissue.

In particular the lipophilicity of the drug is important since the drug must diffuse across the cell membrane and an optimum partition coefficient is usually observed for passive diffusion processes.

Carrier-mediated transport

This form of transport involves specific carrier proteins present in the cell membranes. Carrier-mediated transport can either act with a concentration gradient (facilitated diffusion) or against a concentration gradient (active absorption). For active absorption, as the transport is working against a concentration gradient, energy is required.

Any molecules, including drug molecules, which are similar to the natural substrate of the carrier protein are transported across the cell membrane. As this process involves a carrier protein, the mechanism is saturable at high concentrations and uptake via this route can be inhibited by competing substrates.

Endocytosis

This process involves internalisation of substances by engulfment by the cell membrane which forms membrane-based vesicles within the cell, known as endosomes. This allows larger molecules or particulates to enter the cell. There are several types of endocytosis:

- Receptor-mediated endocytosis: substances interact with specific surface receptors. As this involves receptors, the process is saturable. Drugs bind to receptors on the surface of the cell. This promotes invagination and vesicle formation in the cell. Within these vesicles, known as endosomes, the contents are subjected to low-pH conditions and digestive enzymes which can result in drug degradation/inactivation.

Tips

Fick's first law of diffusion states that the amount of a solute, for example a drug in solution, passing across a unit area, for example of the lipid bilayer of the epithelial barrier (flux, J, units: $kg\,m^{-2}\,s^{-1}$), is proportional to the concentration difference across this unit area (dC/dx, units: $kg\,m^{-4}$). The proportional constant is D (units: $m^2\,s^{-1}$) and the partition coefficient of the drug is K.

$$J = -DK(dC/dx)$$

The minus sign in this equation stems from the fact that diffusion occurs along the concentration gradient, i.e. from higher concentration of the solute to lower concentration. This equation applies to steady-state conditions. Diffusion is discussed in more depth in Chapter 5.

Tips

Examples of carrier-mediated drug transport

- Facilitated diffusion: riboflavin and vitamin B_6 are absorbed by a facilitated diffusional transport.
- Active absorption: levodopa is absorbed by active absorption via amino acid transporters.

- Adsorptive endocytosis: this involves non-specific interactions with the cell surface receptors and therefore is non-saturable.
- Pinocytosis: this involves the uptake of solutes and single molecules. Large soluble macromolecules can be taken up by this process. This is a non-specific process that goes on continually in all cell types.
- Phagocytosis: with this process larger particulates may be taken up. Only specialised cells of the reticuloendothelial system (also known as the mononuclear phagocyte system) are capable of phagocytosis. This includes cells such as blood monocytes and macrophages.

Pore transport

Very small molecules may also be taken up through aqueous pores that are present in some cell membranes. These are ~0.4 nm in diameter so this transport mechanism is very restrictive. Only very small hydrophilic drugs can enter cells via this route.

Paracellular route

Drugs can also cross epithelia through gaps (known as gap junctions) between the cells. This route is governed by passive diffusion and small hydrophilic molecules can pass through these gap junctions. Transport across the epithelia can be enhanced using penetration enhancers which can damage the gap junctions; however possible toxicity implications should be considered with such methods.

Efflux

Substances can also be pushed back out of cells by an energy-dependent efflux system. There are various apical transmembrane proteins which can transport drugs out of the cell. Drugs that are subjected to efflux processes include cytotoxic drugs such as taxol, steroids, immunosuppressants and antibiotics.

Efflux is a major concern in the development of antimicrobial resistance. The genetic information for efflux pumps can be contained within chromosomes and/or plasmids. This allows for the efflux pump genes to be passed to various bacterial species. Expression of several efflux pumps in bacteria can lead to multidrug resistance.

KeyPoints

- The role of the drug delivery systems is to allow the effective, safe, and reliable application of the drug to the patient.
- To achieve this aim the drug must reach its target site.
- The system must be able to be produced in a technically feasible way and the quality of the formulation process must be assured.

Summary

No matter how dosage forms are classified, the role of the drug delivery systems is to

allow the effective, safe, and reliable application of the drug to the patient.

For the development of dosage forms the formulation scientist needs to optimise the bioavailability of the drug. This means the delivery systems should allow and facilitate the drug to reach its target site in the body. For example, a tablet formulation containing an antihypertensive drug must disintegrate in the gastrointestinal tract, the drug needs to dissolve and the dissolved drug needs to permeate across the mucosal membrane of the gastrointestinal tract into the body.

Whilst some drugs are meant to act locally, e.g. in the oral cavity, in the gastrointestinal tract, in the eye or on the skin, nevertheless the prime role of the drug delivery system is to allow the drug to reach its target site.

Tip

If a drug is in the gastrointestinal tract it is still outside the body.

Another role of the delivery systems is to allow the safe application of the drug. This includes that the drug in the formulation must be chemically, physically and microbiologically stable. Side-effects of the drug and drug interactions should be avoided or minimised by the use of suitable drug delivery systems. The delivery systems also need to improve the patient's compliance with the pharmacotherapy by the development of convenient applications. For example, one can improve patient compliance by developing an oral dosage form where previously only parenteral application was possible.

Tip

Some confusion may arise from the use of the expression targeted drug delivery systems. In this book we define targeted delivery system as systems that allow selective targeting of the drug to a specific tissue, organ or specific cells inside the body to achieve a targeted drug *action*. If the *release* of the drug from the dosage form is targeted to a specific organ, these systems may be better called topical delivery systems (although some authors define only dermal application of dosage forms as being topical).

Finally, the delivery system needs to be reliable and its formulation needs to be technically feasible. This means the pharmaceutical quality of the delivery systems needs to be assured, drug release from the system needs to be reproducible and the influence of the body on drug release should be minimised (for example, food effects after oral administration). However, for any application of a drug delivery system on the market, the dosage form needs to be produced in large quantities and at low costs to make affordable medicines available. Therefore, it is also necessary to investigate the feasibility of the developed systems to be scaled up from the laboratory to the production scale. Figure 1.7 summarises the key attributes to be optimised to develop a drug into a medicine.

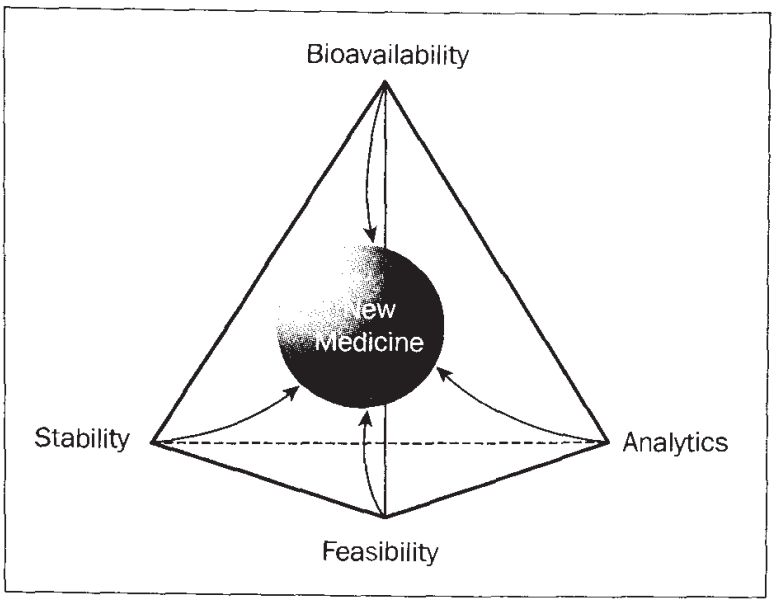


Figure 1.7 Key attributes that need to be optimised to develop a drug into a medicine.

Self-assessment

After having read this chapter you should be able to:

- differentiate dosage forms according to their physical state and to give examples for each category
- differentiate dosage forms according to their route of administration and to list examples for each category
- differentiate dosage forms according to their drug release and to list examples for each category
- describe and explain plasma concentration versus time profiles of immediate-release oral dosage forms
- describe and explain plasma concentration versus time profiles of delayed-release oral dosage forms
- describe and explain plasma concentration versus time profiles of sustained-release oral dosage forms
- describe and explain plasma concentration versus time profiles of controlled-release oral dosage forms
- discuss the therapeutic range of a drug and how it is linked to the plasma concentration versus time profiles of oral dosage forms
- compare and contrast targeted and non-targeted drug release
- identify the essential features of transcellular and paracellular absorption via:
  - passive diffusion
  - carrier-mediated transport
  - endocytosis
  - paracellular absorption
  - efflux
- discuss the key attributes to be optimised to develop a drug into a medicine.

Questions

1. **Indicate which one of the following statements is not correct:**

a. The drug delivery system can play a vital role in controlling the pharmacological effect of the drug.

b. The drug delivery system can influence the pharmacokinetic profile of the drug, the rate of drug release, the site and duration of drug action and subsequently the side-effect profile.

c. An optimal drug delivery system ensures that the active drug is available at the site of action for the correct time and duration.

d. The drug concentration at the appropriate site should be below the minimal effective concentration (MEC).

e. The concentration interval between the MEC and the minimal toxic concentration (MTC) is known as the therapeutic range.

2. **Indicate which one of the following statements is not correct:**

a. A simple emulsion contains two liquid phases (oil and water).

b. In a water-in-oil emulsion, the oil phase is dispersed and the water phase is continuous.

c. A simple suspension contains a liquid and a solid phase.

d. In a suspension the solid phase is dispersed and the liquid phase is continuous.

e. In most multiphase dosage forms one or more phases are dispersed, whilst other phases are continuous.

3. **Indicate which one of the following statements is not correct:**

a. Dispersing one phase into the other will lead to a larger interfacial area between the two phases.

b. A larger interfacial area between the two phases leads to an increased interfacial free energy, according to the relationship: $G_i = A\gamma$.

c. In the equation $G_i = A\gamma$, G_i is the interfacial free energy of the system.

d. In the equation $G_i = A\gamma$, A is the interfacial area between the dispersed phase and the continuous phase.

e. In the equation $G_i = A\gamma$, γ is the surface tension of the continuous phase.

4. **Indicate which one of the following statements is not correct:**

a. The most important route of drug administration into the body is through mucosal membranes.

b. Mucosal membranes are a stronger barrier to drug uptake than the skin.

c. The mucosal membranes of the small intestine are specialised sites for absorption.

d. There are many mucosal membranes that can be used for drug administration.

e. Absorption of drugs through the mucosal membranes of the gastrointestinal tract allows for oral drug delivery.

5. Indicate which one of the following statements is not correct:

a. Drugs that are taken up into the body through the gastrointestinal mucosa will be transported to the liver via the portal vein before going into general circulation.

b. If the drug is susceptible to metabolic degradation in the liver, this may considerably enhance the activity of the drug. This phenomenon is known as the hepatic first-pass effect.

c. The rectal route may also show varying degrees of the first-pass effect.

d. In other routes of administration (intravenous, vaginal, nasal, buccal and sublingual) the drug is distributed in the body before reaching the liver.

e. Whilst the liver is the main metabolic organ of the body, metabolism may also take place in the gastrointestinal lumen and indeed in the mucosal membranes.

6. Indicate which one of the following statements is not correct:

a. Many dosage forms are designed to release the drug immediately after administration. This is useful if a fast onset of action is required for therapeutic reasons.

b. The onset of action is very fast for intravenous injections and infusions and a pharmacological effect may be seen in a matter of seconds after administration.

c. The onset of action is fast for oral delivery of immediate-release dosage forms, such as simple tablets, and a pharmacological effect may be seen in a matter of minutes to hours.

d. If the drug has a long biological half-life, the time interval between administrations may be short, requiring frequent dosing and potentially leading to low patient compliance and suboptimal therapeutic outcome.

e. Uptake of a drug through the mucosal membranes may be due to passive diffusion or by receptor-mediated active transport mechanisms.

7. Indicate which one of the following statements is not correct:

a. Delayed-release dosage forms can be defined as systems formulated to release the active ingredient at a time other than immediately after administration.

b. Colon-specific dosage forms are developed for the treatment of local and systemic diseases in the colon, including colorectal cancer and Crohn's disease.

c. In the plasma concentration versus time profile of a delayed-release oral dosage form C_{max} (but not T_{max}) is strongly dependent on the gastric emptying times.

d. Delayed-release systems can be used to protect the drug from degradation in the low-pH environment of the stomach.
e. Delayed-release systems can be used to protect the stomach from irritation by the drug.

8. Indicate which one of the following statements is not correct:
a. The release kinetics of the drugs from sustained-release matrix systems often follows a first-order kinetics.
b. The release kinetics of the drugs from sustained-release reservoir systems often follows a zero-order kinetics.
c. If the release of the drug from the dosage form is sustained such that the release takes place throughout the entire gastrointestinal tract, one can reduce C_{max} and prolong the time interval of drug concentration in the therapeutic range.
d. The use of sustained-release dosage forms may reduce the frequency of dosing, for example from three times a day to once a day.
e. Sustained-release dosage forms can achieve their release characteristics by the use of suitable polymers.

9. Indicate which one of the following statements is not correct:
a. In contrast to sustained-release forms, controlled-release systems are designed to lead to predictable and constant plasma concentrations, independently of the biological environment of the application site.
b. Controlled-release systems are controlling the drug concentration in the body, not just the release of the drug from the dosage form.
c. Controlled-release systems are used in a variety of administration routes, including transdermal, oral and vaginal administration.
d. In contrast to sustained-release forms, in controlled-release systems the dose is of less importance than the release rate from the therapeutic system.
e. Controlled-release systems are target-specific, which means they 'exclusively' deliver the drug to the target organ inside the body.

10. Indicate which one of the following statements is not correct:
a. In drug absorption, passive diffusion involves the diffusion of drugs across the cell membrane and is driven by a concentration gradient, with drugs moving from high to low concentration.
b. Carrier-mediated transport involves specific carrier proteins present in the cell membranes and can act either with a concentration gradient (facilitated diffusion) or against a concentration gradient (active absorption).
c. Endocytosis involves internalisation of substances by engulfment by the cell membrane which forms membrane-based vesicles within the cell, known as liposomes.

d. Some drugs can cross epithelia through gaps between the cells. This route is governed by passive diffusion and small hydrophilic molecules can pass through these gap junctions.

e. Drugs that are subjected to efflux processes include cytotoxic drugs such as taxol, steroids, immunosuppressants and antibiotics.

Further reading

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Attachment A-7

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BIOASSAY OF

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FOR POSSIBLE CARCINOGENICITY

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Public Health Service
National Institutes of Health



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Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health
Bethesda, Maryland 20014

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BIOASSAY OF
5-AZACYTIDINE
FOR POSSIBLE CARCINOGENICITY

Carcinogenesis Testing Program
Division of Cancer Cause and Prevention
National Cancer Institute
National Institutes of Health

CONTRIBUTORS: This report presents the results of the bioassay of 5-azacytidine for possible carcinogenicity conducted for the Carcinogenesis Testing Program, Divison of Cancer Cause and Prevention, National Cancer Institute (NCI), Bethesda, Maryland. The bioassay was conducted by Southern Research Institute, Birmingham, Alabama, initially under direct contract to NCI and currently under a subcontract to Tracor Jitco, Inc., prime contractor for the NCI Carcinogenesis Testing Program.

The experimental design and doses were determined by Drs. D. P. Griswold[1], J. D. Prejean[1], E. K. Weisburger[2], and J. H. Weisburger[2,3]. Ms. J. Belzer[1] and Mr. I. Brown[1] were responsible for the care and treatment of the laboratory animals. Data management and retrieval were performed by Ms. C. A. Dominick[1]. Histopathologic examinations were performed by Drs. S. D. Kosanke[1] and J. C. Peckham[1], and the diagnoses included in this report represent their interpretation. The neoplasms and chemical-related hyperplastic lesions were reviewed by Dr. J. F. Hardisty[4], who also prepared the interpretive pathology narrative included in this report.

Animal pathology tables and survival tables were compiled at EG&G Mason Research Institute[5]. Statistical analyses were performed by Dr. J. R. Joiner[6], using methods selected for the bioassay program by Dr. J. J. Gart[7]. Chemicals used in this bioassay were analyzed by Drs. P. Lim[8], A. Cheung[8], and R. Yee[8], and the analytical results were reviewed by Dr. S. S. Olin[6].

This report was prepared at Tracor Jitco[6] under the direction of NCI. Those responsible for the report at Tracor Jitco were

Dr. Marshall Steinberg, Director of the Bioassay Program; Drs. J. F. Robens and R. W. Fogleman, toxicologists; Dr. R. L. Schueler, pathologist; Ms. M. S. King and Mr. W. D. Reichardt, technical writers; and Dr. E. W. Gunberg, technical editor, assisted by Ms. Y. E. Presley.

The statistical analysis was reviewed by members of the Mathematical Statistics and Applied Mathematics Section of NCI[7]: Dr. John J. Gart, Mr. Jun-mo Nam, Dr. Hugh M. Pettigrew, and Dr. Robert E. Tarone.

The following other scientists at the National Cancer Institute were responsible for evaluating the bioassay experiment, interpreting the results, and reporting the findings:

Dr. Kenneth C. Chu
Dr. Cipriano Cueto, Jr.
Dr. J. Fielding Douglas
Dr. Dawn G. Goodman
Dr. Richard A. Griesemer
Mr. Harry A. Milman
Dr. Thomas W. Orme
Dr. Robert A. Squire[9]
Dr. Jerrold M. Ward

---

[1]Southern Research Institute, 2000 Ninth Avenue South, Birmingham, Alabama.

[2]Carcinogenesis Testing Program, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

[3]Now with the Naylor Dana Institute for Disease Prevention, American Health Foundation, Hammond House Road, Valhalla, New York.

[4]Experimental Pathology Laboratories Inc., P.O. Box 474, Herndon, Virginia.

[5]EG&G Mason Research Institute, 1530 East Jefferson Street, Rockville, Maryland.

[6]Tracor Jitco, Inc., 1776 East Jefferson Street, Rockville, Maryland.

[7]Mathematical Statistics and Applied Mathematics Section, Biometry Branch, Field Studies and Statistics, Division of Cancer Cause and Prevention, National Cancer Institute, National Institutes of Health, Bethesda, Maryland.

[8]Stanford Research Institute, Menlo Park, California.

[9]Now with the Division of Comparative Medicine, Johns Hopkins University, School of Medicine, Traylor Building, Baltimore, Maryland.

SUMMARY

A bioassay of 5-azacytidine for possible carcinogenicity was conducted by administering the test chemical by intraperitoneal injection to Sprague-Dawley rats and B6C3F1 mice.

Groups of 35 rats of each sex were administered 5-azacytidine at one of two doses, either 2.6 or 5.2 mg/kg body weight, in buffered saline three times per week for 34 weeks, and were then observed for 46 or 47 weeks. Controls consisted of groups of 15 rats of each sex that received injections of buffered saline (vehicle controls) and 15 rats of each sex that were untreated (untreated controls). All surviving rats were killed at 80 or 81 weeks.

Groups of 35 mice of each sex were administered the chemical at one of two doses, either 2.2 or 4.4 mg/kg body weight, in buffered saline three times per week for 52 weeks, and were then observed for 29 or 30 weeks. Controls consisted of groups of 15 mice of each sex that received injections of buffered saline (vehicle controls) and 15 mice of each sex that were untreated (untreated controls). All surviving mice were killed at 81 or 82 weeks.

5-Azacytidine was toxic to the animals in this bioassay, since mean body weights of both treated rats and treated mice were lower than those of the corresponding vehicle controls, and since none of the high-dose male and female rats and high-dose female mice lived to the end of the bioassay. In treated male and female rats and male mice, survival was inadequate for meaningful statistical analyses of the incidences of tumors.

Only one male and three female high-dose rats had tumors, and none of the tumors in the low-dose group of either sex were present at a significantly increased incidence using any of the statistical tests. Bone-marrow atrophy was present in both treated groups of both sexes of rats.

Only five high-dose male mice and one high-dose female mouse had neoplasms. In low-dose female mice, however, lymphocytic and granulocytic neoplasms of the hematopoietic system occurred in 17 animals, even though only 54% survived until week 81. Granulocytic neoplasms were observed in 10/29 low-dose female mice, but in no other group, and were significant ($P = 0.010$) compared with the vehicle controls. The incidence of combined lymphoma and granulocytic neoplasms was highly significant in the low-dose females (vehicle controls 0/14, low-dose 17/29, $P < 0.001$). No tumors were observed at a significant incidence in male mice. Bone-marrow atrophy was present in high-dose female mice.

It is concluded that under the conditions of this bioassay, the short life span and short duration of treatment of Sprague-Dawley rats of either sex and of male B6C3F1 mice precluded evaluation of the carcinogenicity of 5-azacytidine in these groups; however, the induction of tumors of the hematopoietic system in female B6C3F1 mice was associated with the administration of 5-azacytidine.

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I. INTRODUCTION

5-Azacytidine (CAS 320-67-2; NCI C01569), a synthetic analogue of cytidine, has been used as an investigational anticancer drug in the United States since 1970 (Von Hoff et al., 1976). Chemical synthesis was first reported in 1964 by a Czech group, and later the chemical was isolated as an antibiotic from a culture filtrate of Streptoverticillium ladakanus (Heidelberger, 1973).

The exact mechanism of action of 5-azacytidine is not known. It is rapidly phosphorylated in mammalian tissues and incorporated into both RNA and DNA. By disrupting the processes of translation of nucleic acid sequences into protein, it inhibits the synthesis of protein. Moreover, by inhibiting orotodylic acid decarboxylase, the chemical also affects de novo pyrimidine synthesis. 5-Azacytidine is rapidly cleared from plasma, is concentrated in lymphatic tissues, and is rapidly excreted in the urine as both unchanged drug and metabolites (Carter and Slavik, 1976).

5-Azacytidine was selected for screening in the carcinogenesis program in an attempt to evaluate the carcinogenicity of certain drugs that may be used in humans for prolonged periods.

II. MATERIALS AND METHODS

A. Chemical

5-Azacytidine, which is a common name for 4-amino-1-beta-D-ribofuranosyl-1,3,5-triazine-2(1H)-one, was obtained as a single batch (Lot No. AP-V-128) from Ash-Stevens, Inc., Detroit, Michigan, by the Drug Development Branch, Division of Cancer Treatment, National Cancer Institute (NCI).

The identity and purity of the chemical were confirmed in analyses at Stanford Research Institute. Elemental analyses (C, H, N, O) were correct for $C_8H_{12}N_4O_5$, the molecular formula of 5-azacytidine. Infrared, ultraviolet, and nuclear magnetic resonance spectra were as expected for this chemical and were identical to spectra of a reference standard. No free 5-azacytosine or D-ribose was detected by paper chromatography. On the basis of these results, the purity was estimated to be > 99%.

The powdered 5-azacytidine was stored at 5°C in small bottles enclosed in sealed plastic bags containing Drierite®.

B. Dosage Preparation

Concentrations of 5-azacytidine of 0.1 or 0.2% for rats and 0.02 or 0.04% for mice were prepared in buffered saline (pH 6.9) for intraperitoneal injection of the chemical. Aqueous solutions

were not stored, because they are unstable at room temperature. The drug and the vehicle were mixed in a 10-ml glass Potter-Elvehjem tissue grinder with a Teflon pestle. Fresh solutions in exact amounts for each administration were prepared preceding injection.

C. Animals

Female Sprague-Dawley rats and male and female Swiss mice were used in subchronic studies.

Sprague-Dawley rats and B6C3F1 mice of both sexes, obtained through contracts of the Division of Cancer Treatment, NCI, were used in chronic studies. The Sprague-Dawley rats were obtained from Charles River Breeding Laboratories, Inc., Wilmington, Massachusetts, and the B6C3F1 mice were obtained from Charles River Laboratories and from A. E. Schmidt, Madison, Wisconsin. On arrival at the laboratory, the male rats were 30 days old, the female rats were 37 days old, and the mice were all 30 days old. The animals were quarantined for an acclimation period (rats for 5 days, mice for 4-5 days), assigned to control and treated groups, and earmarked for individual identification.

D. Animal Maintenance

All animals were housed in temperature- and humidity-controlled

rooms. The temperature range was 20-24°C, and the relative humidity was maintained at 40-60%. There were 15 changes of room air per hour. Air was passed through both intake and exhaust fiberglass roughing filters. In addition to natural light, illumination was provided by fluorescent light for 9 hours per day. Wayne® Lab Blox (Allied Mills, Inc., Chicago, Ill.) and water were supplied daily and were available ad libitum.

Rats were housed five per cage and mice seven per cage in solid-bottom stainless steel cages (Hahn Roofing and Sheet Metal Co., Birmingham, Ala.). The bottoms of the rat cages were lined with Iso-Dri® hardwood chips (Carworth, Edison, N.J.) and cage tops were covered with disposable filter bonnets beginning at week 26; mouse cages were provided with Sterolit® clay bedding (Englehard Mineral and Chemical Co., New York, N.Y.). Bedding was replaced once per week; cages, water bottles, and feeders were sanitized at 82°C once per week; and racks were cleaned once per week.

The rats and mice were housed in separate rooms. Control animals were housed with respective treated animals. Animals treated with 5-azacytidine were maintained in the same rooms as animals of the same species being treated with the following chemicals:

RATS

Gavage Studies

cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate (phenesterin) (CAS 3546-10-9)
estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate) (estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride (MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGDR) (CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester) hydrochloride (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione (ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine hydrochloride (phenoxybenzamine) (CAS 63-92-3)
N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)
adriamycin (CAS 23214-92-8)

MICE

Feed Studies

4-acetyl-N-((cyclohexylamino)carbonyl)benzenesulfonamide (acetohexamide) (CAS 968-81-0)
anthranilic acid (CAS 118-92-3)
1-butyl-3-(p-tolylsulfonyl)urea (tolbutamide) (CAS 64-77-7)
4-chloro-N-((propylamino)carbonyl)benzenesulfonamide (chlorpropamide) (CAS 94-20-2)
5-(4-chlorophenyl)-6-ethyl-2,4-pyrimidinediamine (pyrimethamine) (CAS 58-14-0)
2,6-diamino-3-(phenylazo)pyridine hydrochloride (phenazopyridine hydrochloride) (CAS 136-40-3)
ethionamide (CAS 536-33-4)
L-tryptophan (CAS 73-22-3)

N-9H-fluoren-2-ylacetamide (CAS 53-96-3)
N-(p-toluenesulfonyl)-N′-hexamethyleniminourea
(tolazamide) (CAS 1156-19-0)
1-phenethylbiguanide hydrochloride (phenformin) (CAS 114-86-3)
pyrazinecarboxamide (pyrazinamide) (CAS 98-96-4)
4,4′-sulfonyldianiline (dapsone) (CAS 80-08-0)
4,4′-thiodianiline (CAS 139-65-1)
ethionamide (CAS 536-33-4)

Gavage Studies

cholesterol (p-(bis(2-chloroethyl)amino)phenyl)acetate
(phenesterin) (CAS 3546-10-9)
estradiol bis((p-(bis(2-chloroethyl)amino)phenyl)acetate)
(estradiol mustard) (CAS 22966-79-6)

Intraperitoneal Injection Studies

4′-(9-acridinylamino)methansulfon-m-aniside monohydrochloride
(MAAM) (NSC 141549)
acronycine (CAS 7008-42-6)
beta-2′-deoxy-6-thioguanosine monohydrate (beta-TGDR)
(CAS 789-61-7)
1,4-butanediol dimethanesulfonate (busulfan) (CAS 55-98-1)
emetine dihydrochloride tetrahydrate (CAS 316-42-7)
3,3′-iminobis-1-propanol dimethanesulfonate (ester)
hydrochloride (CAS 3458-22-8)
(±)-4,4′-(1-methyl-1,2-ethanediyl)bis-2,6-piperazinedione
(ICRF-159) (CAS 21416-87-5)
N,3-bis(2-chloroethyl)tetrahydro-2H-1,3,2-oxazaphosphorin-2-
amine-2-oxide (isophosphamide) (CAS 3778-73-2)
N-(2-chloroethyl)-N-(1-methyl-2-phenoxyethyl)benzylamine
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N-(1-methylethyl)-4-((2-methylhydrazino)methyl)benzamide
monohydrochloride (procarbazine) (CAS 366-70-1)
tris(1-aziridinyl)phosphine sulfide (thio-TEPA) (CAS 52-24-4)
2,4,6-tris(dimethylamino)-s-triazine (CAS 645-05-6)
adriamycin (CAS 23214-92-8)

E. Subchronic Studies

Subchronic studies were conducted in female Sprague-Dawley rats and male and female Swiss mice to estimate the maximum tolerated

doses of 5-azacytidine, on the basis of which low and high doses were determined for administration in the chronic studies. The animals were administered 5-azacytidine by intraperitoneal injection three times per week for 45 days, then observed for an additional 45 days. Five animals of each species were used at each dose, 10 animals were used as untreated controls, and 10 animals were used as vehicle (saline) controls.

In rats, administration of the five doses originally selected (0.13, 0.33, 0.65, 1.3, and 2.6 mg/kg body weight) resulted in no deaths and in no weight depression exceeding the 15% guideline, when treated animals were compared with untreated controls. A second study was performed using doses of 2.6, 5.2, 10.4, and 20.8 mg/kg. At 20.8 mg/kg, three animals died in week 4 and all died by week 6. Two animals receiving 10.4 mg/kg died, one in week 6 and one in week 8. At doses of 2.6 or 5.2 mg/kg, no deaths occurred, no lesions were observed, and weight depression did not exceed the 15% guideline. The low and high doses for rats were set at 2.6 and 5.2 mg/kg for the chronic studies.

In mice, the subchronic study was initially conducted in males at doses of 0.22, 0.6, 1.1, 2.2, and 4.4 mg/kg. No deaths attributable to drug toxicity and no weight depression exceeding the 15% guideline resulted. A second study was performed in females, using doses of 4.4, 8.8, 17.6, and 35.2 mg/kg. All animals

receiving 17.6 or 35.2 mg/kg died by week 6, and 3/5 at 8.8 mg/kg died by week 8; however, there were no deaths at 4.4 mg/kg. Weights of animals surviving to the end of the study were similar to those of controls, and no lesions were seen at necropsy. The low and high doses for mice were set at 2.2 and 4.4 mg/kg for the chronic studies.

F. Designs of Chronic Studies

The designs of the chronic studies are shown in tables 1 and 2. Treatment of the rats was terminated at week 34, due to excessive mortality.

G. Clinical and Pathologic Examinations

All animals were observed twice daily for signs of toxicity, and those that were moribund were killed and necropsied. Rats and mice were weighed individually each week for 2 months and every 2 weeks for the remainder of the study. Palpation for masses was carried out at each weighing.

The pathologic evaluation consisted of gross and microscopic examination of major tissues, major organs, and all gross lesions from killed animals and from animals found dead. The following tissues were routinely examined microscopically: skin, muscle, lungs and bronchi, trachea, bone and bone marrow, spleen, lymph

Table 1. Design of Chronic Studies of 5-Azacytidine in Rats

Sex and Treatment Group	Initial No. of Animals[a]	5-Azacytidine Dose[b] (mg/kg)	Time on Study: Treated[c] (weeks)	Time on Study: Untreated (weeks)
Male				
Untreated-Control	15	0		81
Vehicle-Control	15	0[d]	34	46-47
Low-Dose	35	2.6	34	46
High-Dose	35	5.2	34	10[e]
Female				
Untreated-Control	15	0		81
Vehicle-Control	15	0[d]	34	47
Low-Dose	35	2.6	34	46
High-Dose	35	5.2	34	11[e]

[a]Male rats and their controls were 35 days of age when placed on study; females were 42 days of age.

[b]5-Azacytidine was administered in buffered saline by intraperitoneal injection three times per week at a volume of 0.25 ml/100 g body weight. Doses were based on individual weights.

[c]Treatment terminated at 34 weeks rather than at 52 weeks because of high mortality in the high-dose groups.

[d]Vehicle controls received only buffered saline solution, at the same volume as treated rats.

[e]The remaining high-dose males were killed at week 44 and high-dose females at week 45.

Table 2. Design of Chronic Studies of 5-Azacytidine in Mice

Sex and Treatment Group	Initial No. of Animals[a]	5-Azacytidine Dose[b] (mg/kg)	Time on Study: Treated (weeks)	Time on Study: Untreated (weeks)
Male				
Untreated-Control	15	0		81-82
Vehicle-Control	15	0[c]	52	29-30
Low-Dose	35	2.2	52	29
High-Dose	35	4.4	52	29
Female				
Untreated-Control	15	0		82
Vehicle-Control	15	0[c]	52	29-30
Low-Dose	35	2.2	52	29
High-Dose	35	4.4	52	9[d]

[a]All animals were 38 days of age when placed on study.

[b]5-Azacytidine was administered in buffered saline by intraperitoneal injection three times per week at a volume of 1 ml/100 g body weight. Doses were based on the mean weight of the animals in each cage.

[c]Vehicle controls received only buffered saline solution, at the same volume as treated mice.

[d]The remaining high-dose female mice were killed at week 61.

nodes, thymus, heart, salivary gland, liver, gallbladder and bile duct (mice), pancreas, esophagus, stomach, small intestine, large intestine, kidney, urinary bladder, pituitary, adrenal, thyroid, parathyroid, mammary gland, prostate or uterus, testis or ovary, brain, and sensory organs. Peripheral blood smears were prepared from each animal. Occasionally, additional tissues were also examined microscopically. The different tissues were preserved in 10% buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin. Special staining techniques were utilized when indicated for more definitive diagnosis.

A few tissues from some animals were not examined, particularly from those animals that died early. Also, some animals may have been judged to be in such an advanced state of autolysis as to preclude histopathologic evaluation. Thus, the number of animals from which particular organs or tissues were examined microscopically varies, and does not necessarily represent the number of animals that were placed on study in each group.

H. Data Recording and Statistical Analyses

Pertinent data on this experiment have been recorded in an automatic data processing system, the Carcinogenesis Bioassay Data System (Linhart et al., 1974). The data elements include descriptive information on the chemicals, animals, experimental design,

clinical observations, survival, body weight, and individual pathologic results, as recommended by the International Union Against Cancer (Berenblum, 1969). Data tables were generated for verification of data transcription and for statistical review.

These data were analyzed using the statistical techniques described in this section. Those analyses of the experimental results that bear on the possibility of carcinogenicity are discussed in the statistical narrative sections.

Probabilities of survival were estimated by the product-limit procedure of Kaplan and Meier (1958) and are presented in this report in the form of graphs. Animals were statistically censored as of the time that they died of other than natural causes or were found to be missing; animals dying from natural causes were not statistically censored. Statistical analyses for a possible dose-related effect on survival used the method of Cox (1972) for testing two groups for equality and Tarone's (1975) extensions of Cox's methods for testing for a dose-related trend. One-tailed P values have been reported for all tests except the departure from linearity test, which is only reported when its two-tailed P value is less than 0.05.

The incidence of neoplastic or nonneoplastic lesions has been given as the ratio of the number of animals bearing such lesions

at a specific anatomic site (numerator) to the number of animals in which that site is examined (denominator). In most instances, the denominators included only those animals for which that site was examined histologically. However, when macroscopic examination was required to detect lesions prior to histologic sampling (e.g., skin or mammary tumors), or when lesions could have appeared at multiple sites (e.g., lymphomas), the denominators consist of the numbers of animals necropsied.

The purpose of the statistical analyses of tumor incidence is to determine whether animals receiving the test chemical developed a significantly higher proportion of tumors than did the control animals. As a part of these analyses, the one-tailed Fisher exact test (Cox, 1970) was used to compare the tumor incidence of a control group with that of a group of treated animals at each dose level. When results for a number of treated groups (k) are compared simultaneously with those for a control group, a correction to ensure an overall significance level of 0.05 may be made. The Bonferroni inequality (Miller, 1966) requires that the P value for any comparison be less than or equal to 0.05/k. In cases where this correction was used, it is discussed in the narrative section. It is not, however, presented in the tables, where the Fisher exact P values are shown.

The Cochran-Armitage test for linear trend in proportions, with

continuity correction (Armitage, 1971), was also used. Under the assumption of a linear trend, this test determines if the slope of the dose-response curve is different from zero at the one-tailed 0.05 level of significance. Unless otherwise noted, the direction of the significant trend is a positive dose relationship. This method also provides a two-tailed test of departure from linear trend.

A time-adjusted analysis was applied when numerous early deaths resulted from causes that were not associated with the formation of tumors. In this analysis, deaths that occurred before the first tumor was observed were excluded by basing the statistical tests on animals that survived at least 52 weeks, unless a tumor was found at the anatomic site of interest before week 52. When such an early tumor was found, comparisons were based exclusively on animals that survived at least as long as the animal in which the first tumor was found. Once this reduced set of data was obtained, the standard procedures for analyses of the incidence of tumors (Fisher exact tests, Cochran-Armitage tests, etc.) were followed.

When appropriate, life-table methods were used to analyze the incidence of tumors. Curves of the proportions surviving without an observed tumor were computed as in Saffiotti et al. (1972). The week during which an animal died naturally or was sacrificed

was entered as the time point of tumor observation. Cox's methods of comparing these curves were used for two groups; Tarone's extension to testing for linear trend was used for three groups. The statistical tests for the incidence of tumors which used life-table methods were one-tailed and, unless otherwise noted, in the direction of a positive dose relationship. Significant departures from linearity ($P < 0.05$, two-tailed test) were also noted.

The approximate 95 percent confidence interval for the relative risk of each treated group compared to its control was calculated from the exact interval on the odds ratio (Gart, 1971). The relative risk is defined as p_t/p_c where p_t is the true binomial probability of the incidence of a specific type of tumor in a treated group of animals and p_c is the true probability of the spontaneous incidence of the same type of tumor in a control group. The hypothesis of equality between the true proportion of a specific tumor in a treated group and the proportion in a control group corresponds to a relative risk of unity. Values in excess of unity represent the condition of a larger proportion in the treated group than in the control.

The lower and upper limits of the confidence interval of the relative risk have been included in the tables of statistical analyses. The interpretation of the limits is that in

approximately 95% of a large number of identical experiments, the true ratio of the risk in a treated group of animals to that in a control group would be within the interval calculated from the experiment. When the lower limit of the confidence interval is greater than one, it can be inferred that a statistically significant result ($P < 0.025$ one-tailed test when the control incidence is not zero, $P < 0.050$ when the control incidence is zero) has occurred. When the lower limit is less than unity, but the upper limit is greater than unity, the lower limit indicates the absence of a significant result while the upper limit indicates that there is a theoretical possibility of the induction of tumors by the test chemical, which could not be detected under the conditions of this test.

III. RESULTS - RATS

A. Body Weights and Clinical Signs (Rats)

Among males, the mean body weights were lower in the treated groups than in the controls starting at about week 4 of the study, with progressively greater differences occurring as the study progressed, up to approximately week 34 (figure 1). When treatment was discontined at week 34, the mean body weights of the low-dose group markedly increased, as did those of the four remaining high-dose animals. Among females, only the high-dose group had slightly lower mean body weights compared with the controls. Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variations. No other signs of toxicity were recorded for the rats during the bioassay.

B. Survival (Rats)

The Kaplan and Meier curves estimating the probabilities of survival for male and female rats administered 5-azacytidine by intraperitoneal injection at the doses of this experiment, together with those of the controls, are shown in figure 2.

In each sex, the result of the Tarone test for positive dose-related trend in mortality over the period is significant ($P <$

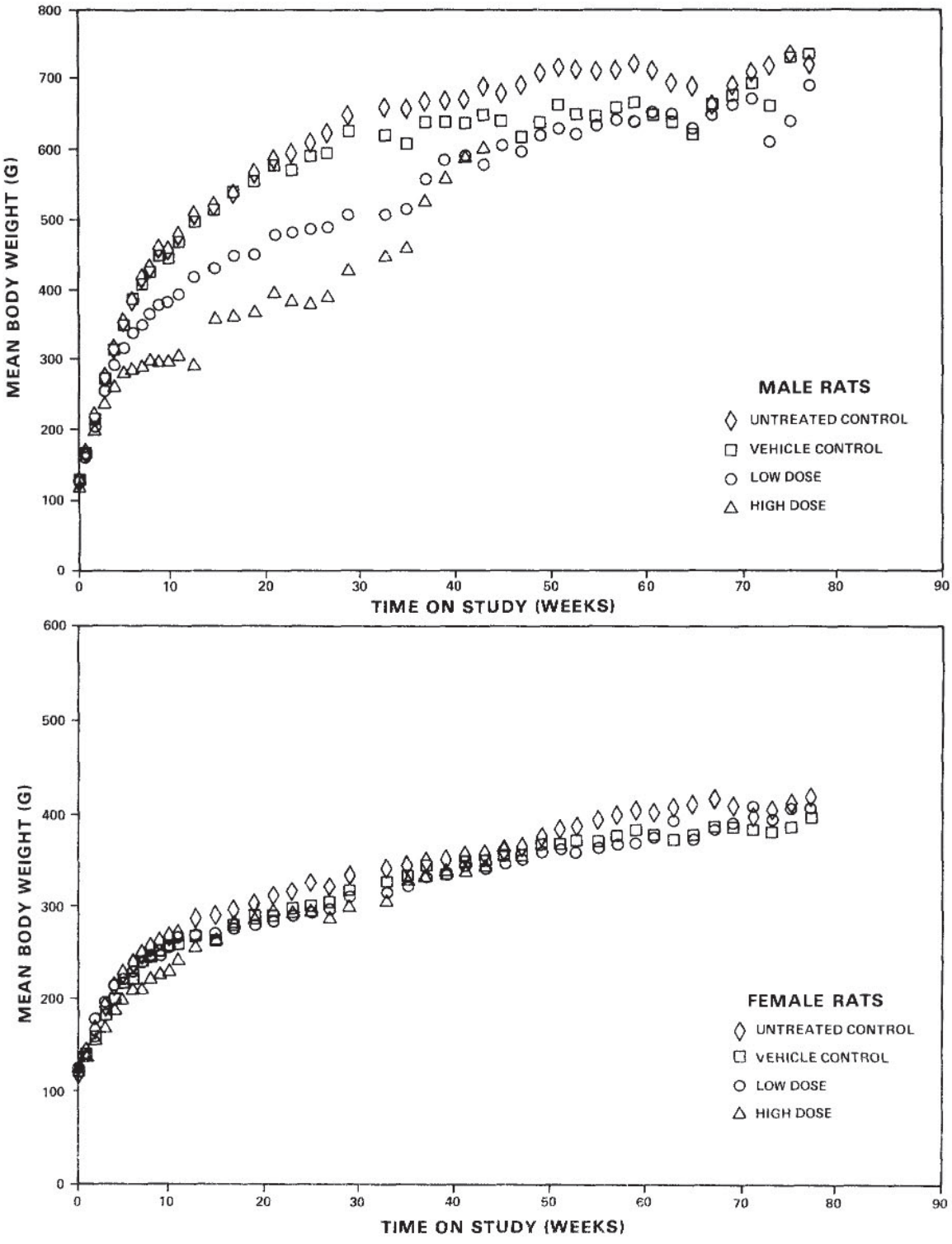


Figure 1. Growth Curves For Rats Treated With 5-Azacytidine

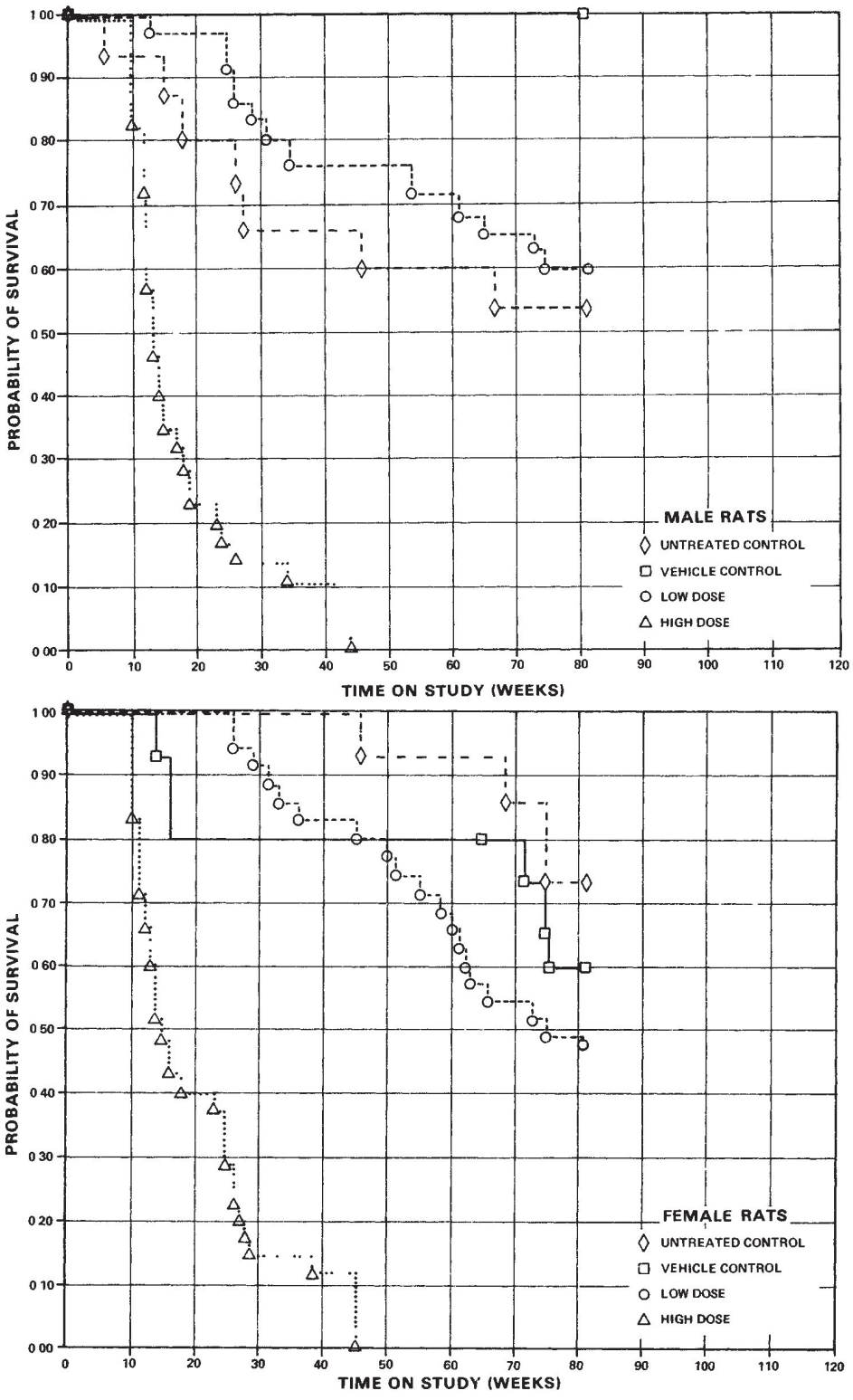


Figure 2. Survival Curves For Rats Treated With 5-Azacytidine

0.001), and a departure from linear trend is present ($P < 0.001$), due to the steep increase in mortality in the high-dose rats, of which all died before the end of the study. The median time on study was only 13 weeks for these rats, and the high mortality at the high dose may have reduced the occurrence of late-developing tumors. In the low-dose rats, 21/35 (60%) of the males and 17/35 (49%) of the females lived to the end of the study at week 81. Time-adjusted analysis was performed on female rats that lived at least 26 weeks, which is the earliest time of an observed tumor. There were 15 such animals in the untreated controls, 1[illegible] in the vehicle controls, 31 in the low-dose group, and 10 in the high-dose group.

C. Pathology (Rats)

Histopathologic findings on neoplasms in rats are summarized in Appendix A, tables A1 and A2; findings on nonneoplastic lesions are summarized in Appendix C, tables C1 and C2.

A variety of neoplasms were seen with approximately equal frequency in the control and treated rats. Neoplasms were seen more frequently in the female rats than in the males. The most frequently observed neoplasms in the females involved the pituitary gland and the mammary gland. Chromophobe adenomas of the pituitary were observed frequently in the control females

only. Benign and malignant mammary gland neoplasms were seen frequently in both control and low-dose female rats. The low incidence of neoplasia in the male and female high-dose groups was probably due to the unusually high number of early deaths that occurred in both high-dose groups.

Several inflammatory and degenerative lesions occurred with approximately equal frequency in treated and control animals. Hepatocellular degeneration and necrosis, usually centrilobular, were present in several of the treated animals and were the only nonneoplastic lesions that appeared to be directly related to exposure to the chemical. None of the control rats had similar hepatic lesions. These lesions were present in 1/31 low-dose and 5/31 high-dose females. Necrotizing liver lesions were also present in 5/31 low-dose and 15/33 high-dose male rats. All of the animals with liver necrosis died prior to the end of this study. Bone-marrow atrophy was present in 5/30 low-dose and 26/33 high-dose males and in 2/30 low-dose and 15/29 high-dose females, but its significance in relation to the early deaths could not be evaluated, due to the lack of clinical pathology data.

In the judgment of the pathologists, the results of this study indicate that the administration of 5-azacytidine at the doses used in this study caused hepatotoxicity and bone-marrow atrophy

in Sprague-Dawley rats. No carcinogenic effect was observed during the shortened life spans of the animals.

D. Statistical Analyses of Results (Rats)

Tables E1 and E2 in Appendix E contain the statistical analyses of the incidences of those primary tumors that were observed in at least two animals with an incidence of at least 5% in either the vehicle-control or low-dose groups. Due to the shortened survival in the high-dose groups, results for these groups are not analyzed in the tables.

Although there was no incidence of chromophobe adenoma of the pituitary in the low-dose female rats (0/30), this tumor is listed in table E2 because of the incidence observed in the vehicle-control group (4/15 [27%]); this incidence gave a significant result in the negative direction. The absence of tumors in the treated group may be accounted for by the high mortality observed in this group. A time-adjusted analysis, eliminating animals that died before week 52 on study, indicated that the difference between the incidences in the treated (0/25) and control (4/14) groups was still significant in the negative direction.

There is no incidence of tumors at any specific site in either sex that is statistically significant in the positive direction.

The absence of such tumors, however, may be due to the abnormally short treatment periods and life spans in the treated animals, rather than to absence of carcinogenicity of the test chemical in the rats.

In each of the 95% confidence intervals of relative risk, shown in the tables, except for the comparison involving the pituitary tumors in female rats, the value of one is included; this indicates the absence of positive significant results. It should also be noted that these intervals have upper limits greater than one, indicating the theoretical possibility of the induction of tumors by 5-azacytidine, which could not be detected under the conditions of this test.

IV. RESULTS - MICE

A. Body Weights and Clinical Signs (Mice)

The mean body weights of the treated mice were dose related throughout the period of the study, and were lower than those of the vehicle controls (figure 3). When treatment was stopped at week 52, some increases in the mean body weights of the remaining animals occurred. A greater difference occurred between body weights of treated and control females than between those of treated and control males. Fluctuations in the growth curve may be due to mortality; as the size of the group diminishes, the mean body weight may be subject to wide variations. No significant signs of toxicity were recorded for the mice during the bioassay.

B. Survival (Mice)

The Kaplan and Meier curves estimating the probabilities of survival for male and female mice administered 5-azacytidine by intraperitoneal injection at the doses of this experiment, together with those of the controls, are shown in figure 4.

In male mice, the result of the Tarone test for positive dose-related trend in mortality over the period is significant ($P < 0.001$), with 20% of the high-dose group, 37% of the low-dose

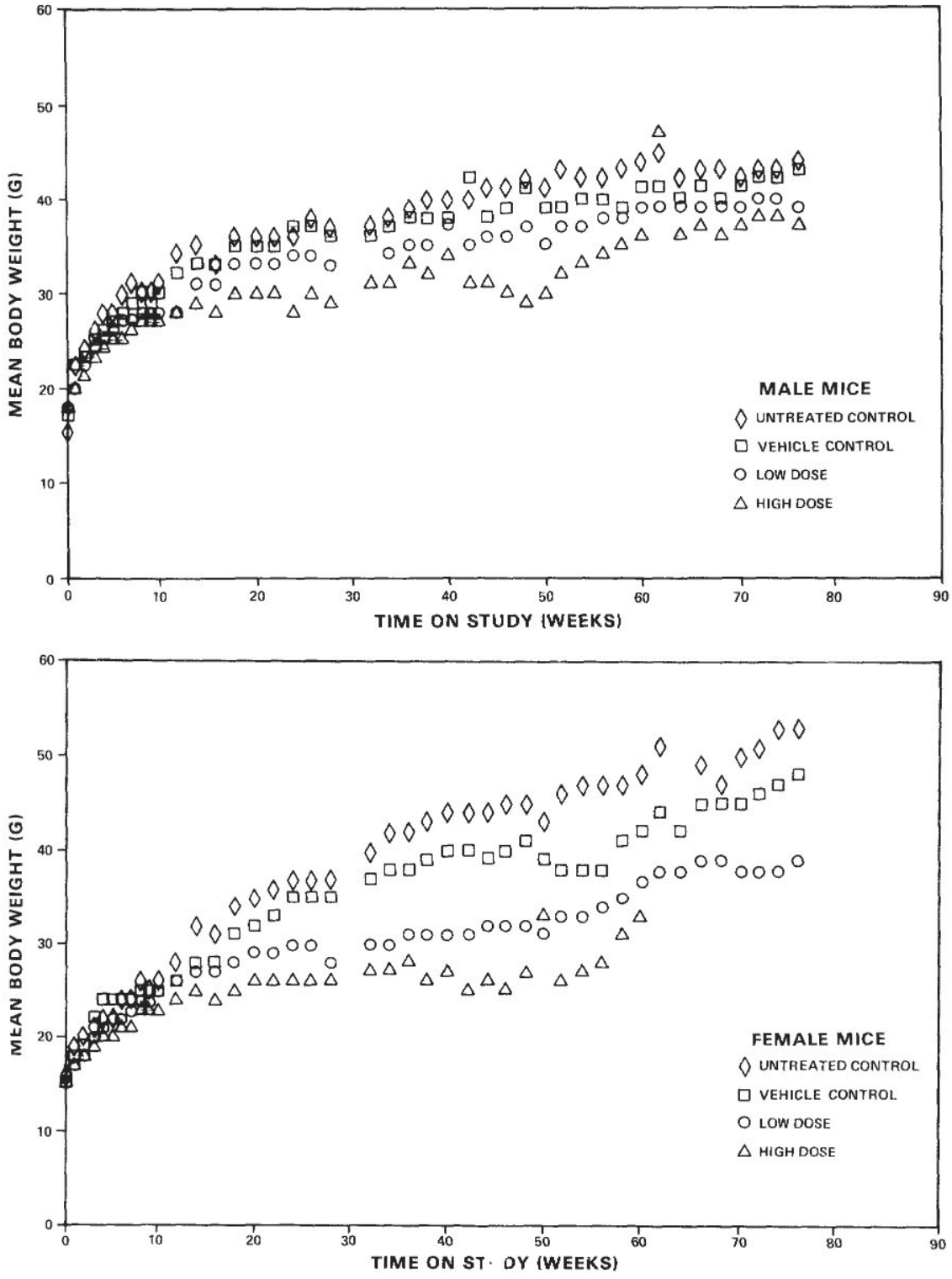


Figure 3. Growth Curves For Mice Treated With 5-Azacytidine

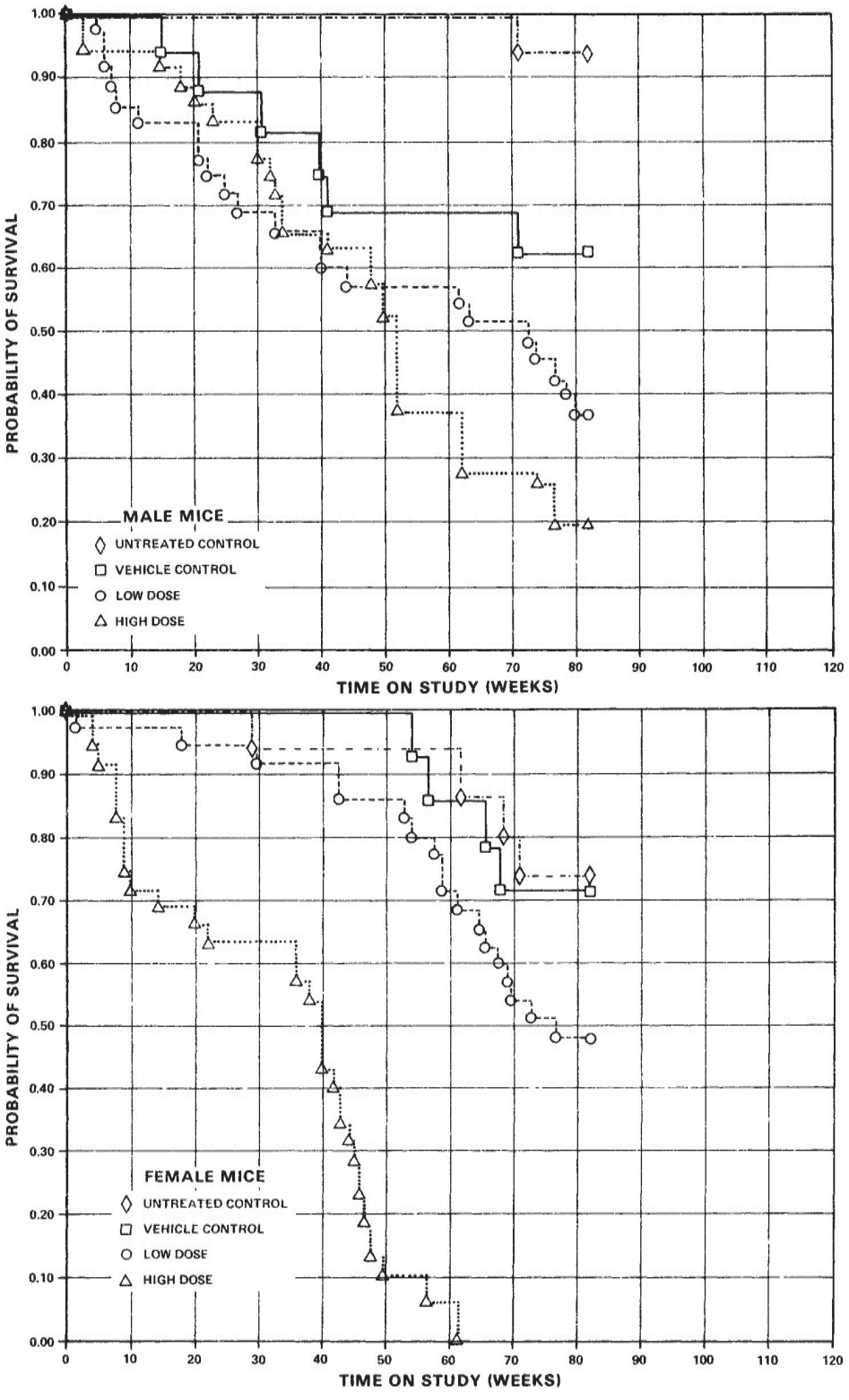


Figure 4. Survival Curves For Mice Treated With 5-Azacytidine

group, 63% of the vehicle controls, and 93% of the untreated controls living to the end of the study. The early deaths of the treated mice may have reduced the occurrence of late-developing tumors.

In female mice, the result of the Tarone test is also significant ($P < 0.001$), and a departure from linear trend is observed ($P < 0.001$), because of the steep increase in mortality among the high-dose mice, of which all died before the end of the study. These early deaths of the high-dose female mice may have suppressed the occurrence of late-developing tumors. Of the low-dose female mice, 49% survived until termination of the study at week 82. In male mice, a time-adjusted analysis was performed on those animals living at least 52 weeks. There were 15 such animals in the untreated controls, 11 in the vehicle controls, and 18 in both the low- and high-dose groups.

C. Pathology (Mice)

Histopathologic findings on neoplasms in mice are summarized in Appendix B, tables B1 and B2; findings on nonneoplastic lesions are summarized in Appendix D, tables D1 and D2.

A variety of neoplasms were present in both treated and control groups. With the exception of the hematopoietic system, neoplasms occurred infrequently and were distributed equally

among the organ systems and groups studied. Several neoplasms of the hematopoietic system involving multiple organs and tissues of the spleen, lymph node, liver, and lung were present in both the male and female mice. Both lymphocytic and granulocytic neoplasms were present in the tissues examined. With the exception of one untreated-control female mouse with malignant lymphoma, all of the hematopoietic neoplasms occurred in treated animals. Hematopoietic neoplasms were diagnosed most frequently in the low-dose groups (male 4/31, female 17/29), and the lower incidence of hematopoietic neoplasms in the high-dose groups (male 3/32, female 0/30) may be the result of the high number of early deaths in these groups. In general, the hematopoietic neoplasms present in the low-dose groups were poorly differentiated, and precise classification of cell type was extremely difficult. The neoplasms observed histologically were classified into the following cell types: malignant lymphoma, lymphocytic type; malignant lymphoma, histiocytic type; malignant lymphoma, undifferentiated type; granulocytic sarcoma; and granulocytic leukemia.

Several inflammatory, degenerative, and proliferative lesions commonly seen in B6C3F1 hybrid mice occurred with approximately equal frequency in treated and control animals. The most common of these lesions were interstitial pneumonia with perivascular

and peribronchiolar lymphocytic hyperplasia and extramedullary hematopoiesis in the spleen. Necrosis of the liver was occasionally observed. Mice that died prior to termination of the study had no consistent lesions, except bone-marrow atrophy in high-dose females, that would account for the early deaths.

In the judgment of the pathologists, the results of this study indicate that the administration of 5-azacytidine at the doses used had a carcinogenic effect upon the hematopoietic system in the B6C3F1 hybrid female mouse. This carcinogenic effect was manifested by the apparent induction of lymphocytic and granulocytic neoplasms in the low-dose female mice. There appeared to be only a slight increase in the number of hematopoietic neoplasms in the high-dose groups; however, the high number of early deaths in this group may have accounted for this observation.

D. Statistical Analyses of Results (Mice)

Tables F1 and F2 in Appendix F contain the statistical analyses of the incidences of those primary tumors that were observed in at least two animals and with an incidence of at least 5% in one or more than one group.

In male mice, there is no incidence of tumors at any specific site that is statistically significant. When time-adjusted

analyses, eliminating animals that died before week 52 of the study, were performed on the incidences of lymphoma of the hematopoietic system, the incidences (vehicle controls 0/11, low-dose 4/18 [22%], high-dose 3/18 [17%]) still remain not significant.

In female mice, the analyses of the incidences of lymphoma show that the Fisher exact comparison of incidences in the low-dose (7/27 [24%]) and vehicle-control (0/14) groups has a probability level of 0.048; however, this probability is above the 0.025 level required by the Bonferroni inequality criterion when the multiple comparison test is applied. Granulocytic leukemia or sarcoma of the hematopoietic system was found exclusively in the low-dose female mice. The Fisher exact test shows that the incidence in the low-dose group (10/29 [34%]) is significantly higher ($P = 0.010$) than that in the vehicle controls (0/14).

The analyses of the combination of lymphoma, granulocytic leukemia, and sarcoma in female mice indicates that the incidence of these tumors in the low-dose group (17/29) differs significantly ($P < 0.001$) from that in the vehicle-control group (0/14), in which no such tumors were seen. The untreated-control group, not shown in the table, was observed to have a 1/15 (7%) incidence of lymphoma. The early mortality in the high-dose group may account for the absence of these tumors in this group. The

incidence of these types of tumors compiled to date in the bioassay studies at this laboratory is 49/265 (19%) in female mice, compared with 17/29 (59%) observed in the low-dose female mice in this study; however, the laboratory results to date show only 1/265 granulocytic sarcoma in vehicle controls compared with 9/29 (31%) in the study. The statistical conclusion is that there is an association between the incidence of hematopoietic tumors and the administration of the low dose of the chemical to female mice.

V. DISCUSSION

The test chemical, 5-azacytidine, was toxic to both the rats and the mice in this bioassay, as demonstrated by (1) the lower mean body weights of the treated animals compared with the controls and (2) the high mortality which occurred during the study. The mean body weights of the treated male rats were much lower than those of the controls during the treatment period of 34 weeks. Because of the high mortality, treatment was discontinued; the low-dose males then gained weight rapidly. Nearly all high-dose animals had died by this time; the median time on study for all high-dose rats was only 13 weeks. Among the low-dose rats, 60% of the male and 49% of the females survived until termination of the study at week 81. Both the low survival and the short duration of treatment prevented evaluation of carcinogenicity of this compound in rats.

Treated mice had lower mean body weights than controls throughout the study, and survival was dose related in both male and female mice. All high-dose females died prior to termination of the study, and only 7 high-dose males, 13 low-dose males, and 17 low-dose females survived to the end of the study at week 81.

Only one male and three female high-dose rats had tumors, and none of the incidences of the tumors in the low-dose group of

either sex were significantly increased using any of the statistical tests. Bone-marrow atrophy was observed in 17% of the low-dose and 79% of the high-dose male rats, in 7% of the low-dose and 52% of the high-dose female rats, and in only 2% of the combined controls. Centrilobular degeneration and necrosis of hepatocytes were observed in 16% of the low-dose and 45% of the high-dose male rats, in 3% of the low-dose and 16% of the high-dose female rats, and in none of the combined controls.

Only five high-dose male mice and one high-dose female mouse had neoplasms. In low-dose female mice, neoplasms of the hematopoietic system including malignant lymphoma, granulocytic sarcoma, and granulocytic leukemia occurred in 17 aniamls, even though only 54% survived until week 81. Malignant lymphoma occurred in greater numbers in the low-dose female mice (7/29) than in the vehicle controls (0/14). The probability level for direct comparison with the vehicle controls was P = 0.048; however, this is above the 0.025 level required by the Bonferroni inequality criterion for significance in multiple comparisons. Granulocytic leukemia or sarcoma was observed in 10/29 low-dose female mice, but in no other group, and 7/29 of the low-dose females died prior to the first observation of this tumor at week 53. The incidence was significant when compared with that of the vehicle-control group (P = 0.010). The incidence of combined lymphoma

and granulocytic neoplasms was highly significant in the low-dose females (vehicle controls 0/14, low-dose 17/29, $P < 0.001$). Early deaths in the high-dose animals may have precluded the observation of neoplasms, and thus, the low incidence of tumors in the high-dose group does not contradict the association with treatment of the tumors observed in the low-dose females. No tumors were observed at a significant incidence in male mice. Hepatocellular necrosis similar to that seen in the rats was occasionally observed, but the occurrence of this lesion does not explain the early deaths. Bone-marrow atrophy was present in 10/29 (34%) of the high-dose female mice.

No other long-term studies of 5-azacytidine have been reported; however, humans treated with the drug have developed leucopenia (lowered total white cell count) and, occasionally, hepatic toxicity (Von Hoff et al., 1976).

It is concluded that under the conditions of this bioassay, the short life span and short duration of treatment of Sprague-Dawley rats of either sex and of male B6C3F1 mice precluded evaluation of the carcinogenicity of 5-azacytidine in these groups; however, the induction of tumors of the hematopoietic system in female B6C3F1 mice was associated with the administration of 5-azacytidine.

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APPENDIX A

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

TABLE A1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS NECROPSIED	14	15	32	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	15	32	33
INTEGUMENTARY SYSTEM				
*SKIN	(14)	(15)	(32)	(33)
PAPILLOMA, NOS			1 (3%)	
SQUAMOUS CELL CARCINOMA			1 (3%)	
*SUBCUT TISSUE	(14)	(15)	(32)	(33)
UNDIFFERENTIATED CARCINOMA			1 (3%)	
BASAL-CELL CARCINOMA			1 (3%)	
SARCOMA, NOS			1 (3%)	
FIBROSARCOMA	1 (7%)			
RESPIRATORY SYSTEM				
NONE				
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(14)	(15)	(32)	(33)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (3%)	
GRANULOCYTIC LEUKEMIA			2 (6%)	
GRANULOCYTIC SARCOMA			1 (3%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				
URINARY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM				
#PITUITARY	(13)	(15)	(29)	(31)
CHROMOPHOBE CARCINOMA		1 (7%)	1 (3%)	
#ADRENAL	(14)	(15)	(31)	(33)
PHEOCHROMOCYTOMA			1 (3%)	
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(14)	(15)	(32)	(33)
ADENOCARCINOMA, NOS			1 (3%)	
FIBROADENOMA				1 (3%)
#TESTIS	(14)	(15)	(28)	(32)
INTERSTITIAL-CELL TUMOR			2 (7%)	
NERVOUS SYSTEM				
#BRAIN	(14)	(15)	(30)	(32)
ASTROCYTOMA	1 (7%)			
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A1. MALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	35	35
NATURAL DEATH@	4		11	20
MORIBUND SACRIFICE	3		3	15
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	8	15	21	
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	1	11	1
TOTAL PRIMARY TUMORS	2	1	14	1
TOTAL ANIMALS WITH BENIGN TUMORS			4	1
TOTAL BENIGN TUMORS			4	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	2	1	9	
TOTAL MALIGNANT TUMORS	2	1	10	
TOTAL ANIMALS WITH SECONDARY TUMORS#				
TOTAL SECONDARY TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				
* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS				
# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN				

TABLE A2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS NECROPSIED	15	15	31	31
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	15	31	31
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(15)	(15)	(31)	(31)
FIBROMA		1 (7%)		
RESPIRATORY SYSTEM				
#LUNG	(15)	(14)	(30)	(30)
ADENOCARCINOMA, NOS, METASTATIC	1 (7%)		2 (7%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(15)	(31)	(31)
LYMPHOCYTIC LEUKEMIA			1 (3%)	
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
NONE				
URINARY SYSTEM				
#KIDNEY	(15)	(13)	(31)	(31)
TUBULAR-CELL ADENOCARCINOMA	1 (7%)			
ENDOCRINE SYSTEM				
#PITUITARY	(14)	(15)	(30)	(27)
CHROMOPHOBE ADENOMA	5 (36%)	4 (27%)		
CHROMOPHOBE CARCINOMA		1 (7%)		

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(15)	(15)	(31)	(31)
ADENOCARCINOMA, NOS	4 (27%)		9 (29%)	1 (3%)
PAPILLARY ADENOCARCINOMA			1 (3%)	1 (3%)
FIBROADENOMA	2 (13%)	3 (20%)	6 (19%)	1 (3%)
#UTERUS	(15)	(15)	(30)	(29)
LEIOMYOSARCOMA			1 (3%)	
ENDOMETRIAL STROMAL POLYP			1 (3%)	
NERVOUS SYSTEM				
#BRAIN	(14)	(14)	(30)	(30)
CHROMOPHOBE CARCINOMA, METASTATI		1 (7%)		
SPECIAL SENSE ORGANS				
*EAR CANAL	(15)	(15)	(31)	(31)
KERATOACANTHOMA			1 (3%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*MESENTERY	(15)	(15)	(31)	(31)
LIPOMA	1 (7%)			
ALL OTHER SYSTEMS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE A2. FEMALE RATS: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	15	35	35
NATURAL DEATH@	1	1	10	22
MORIBUND SACRIFICE	3	5	8	13
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	11	9	17	
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	11	7	15	3
TOTAL PRIMARY TUMORS	13	9	20	3
TOTAL ANIMALS WITH BENIGN TUMORS	8	6	7	1
TOTAL BENIGN TUMORS	8	8	8	1
TOTAL ANIMALS WITH MALIGNANT TUMORS	5	1	11	2
TOTAL MALIGNANT TUMORS	5	1	12	2
TOTAL ANIMALS WITH SECONDARY TUMORS#	1	1	2	
TOTAL SECONDARY TUMORS	1	1	2	
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX B

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

TABLE B1.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	16	35	35
ANIMALS NECROPSIED	15	13	31	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	13	30	30
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(15)	(13)	(29)	(30)
UNDIFFERENTIATED CARCINOMA METAS	1 (7%)			
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (8%)		1 (3%)
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(13)	(31)	(32)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			4 (13%)	2 (6%)
MALIG.LYMPHOMA, HISTIOCYTIC TYPE				1 (3%)
#MEDIASTINAL L.NODE	(15)	(3)	(23)	(20)
UNDIFFERENTIATED CARCINOMA METAS	1 (7%)			
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER	(15)	(13)	(30)	(30)
UNDIFFERENTIATED CARCINOMA	1 (7%)			
HEPATOCELLULAR ADENOMA	1 (7%)	1 (8%)	1 (3%)	1 (3%)
URINARY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM				
*THYROID	(11)	(7)	(24)	(20)
ADENOMA, NOS			1 (4%)	
REPRODUCTIVE SYSTEM				
NONE				
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EAR CANAL	(15)	(13)	(31)	(32)
KERATOACANTHOMA				1 (3%)
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	16	35	35
NATURAL DEATH@		5	18	15
MORIBUND SACRIFICE	1	1	4	13
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	14	10	13	7
ANIMAL MISSING				

@ INCLUDES AUTOLYZED ANIMALS

$ NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B1. MALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	2	6	5
TOTAL PRIMARY TUMORS	2	2	6	6
TOTAL ANIMALS WITH BENIGN TUMORS	1	2	2	2
TOTAL BENIGN TUMORS	1	2	2	3
TOTAL ANIMALS WITH MALIGNANT TUMORS	1		4	3
TOTAL MALIGNANT TUMORS	1		4	3
TOTAL ANIMALS WITH SECONDARY TUMORS#	1			
TOTAL SECONDARY TUMORS	2			
TOTAL ANIMALS WITH TUMORS UNCERTAIN-BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN-PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
\# SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

TABLE B2.

SUMMARY OF THE INCIDENCE OF NEOPLASMS IN FEMALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	14	35	35
ANIMALS NECROPSIED	15	14	29	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	13	29	30
INTEGUMENTARY SYSTEM				
*SUBCUT TISSUE	(15)	(14)	(29)	(30)
BASAL-CELL CARCINOMA				1 (3%)
RESPIRATORY SYSTEM				
#LUNG	(15)	(13)	(28)	(30)
UNDIFFERENTIATED CARCINOMA METAS	1 (7%)			
BASAL-CELL CARCINOMA, METASTATIC			1 (4%)	
ALVEOLAR/BRONCHIOLAR ADENOMA		1 (8%)		
ALVEOLAR/BRONCHIOLAR CARCINOMA		1 (8%)		
SARCOMA, NOS, METASTATIC			1 (4%)	
HEMATOPOIETIC SYSTEM				
*MULTIPLE ORGANS	(15)	(14)	(29)	(30)
MALIG.LYMPHOMA, UNDIFFER-TYPE			1 (3%)	
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE	1 (7%)		4 (14%)	
GRANULOCYTIC LEUKEMIA			1 (3%)	
GRANULOCYTIC SARCOMA			9 (31%)	
#LIVER	(15)	(13)	(29)	(30)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (3%)	
#KIDNEY	(15)	(13)	(29)	(29)
MALIG.LYMPHOMA, LYMPHOCYTIC TYPE			1 (3%)	
CIRCULATORY SYSTEM				
#HEART	(14)	(12)	(28)	(30)
SARCOMA, NOS			1 (4%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM				
#LIVER	(15)	(13)	(29)	(30)
HEPATOCELLULAR ADENOMA	1 (7%)			
LEIOMYOSARCOMA, METASTATIC			1 (3%)	
URINARY SYSTEM				
NONE				
ENDOCRINE SYSTEM				
#ADRENAL	(14)	(13)	(27)	(29)
LEIOMYOSARCOMA, METASTATIC			1 (4%)	
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(15)	(14)	(29)	(30)
ADENOCARCINOMA, NOS			2 (7%)	
#UTERUS	(15)	(13)	(28)	(27)
SARCOMA, NOS			1 (4%)	
LEIOMYOSARCOMA			2 (7%)	
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
*EAR CANAL	(15)	(14)	(29)	(30)
BASAL-CELL CARCINOMA			1 (3%)	
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(15)	(14)	(29)	(30)
LEIOMYOSARCOMA, METASTATIC			1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE B2. FEMALE MICE: NEOPLASMS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
*PLEURA	(15)	(14)	(29)	(30)
LEIOMYOSARCOMA, METASTATIC			1 (3%)	
ALL OTHER SYSTEMS				
NONE				
ANIMAL DISPOSITION SUMMARY				
ANIMALS INITIALLY IN STUDY	15	14	35	35
NATURAL DEATH@	1	1	14	11
MORIBUND SACRIFICE	3	3	4	24
SCHEDULED SACRIFICE				
ACCIDENTALLY KILLED				
TERMINAL SACRIFICE	11	10	17	
ANIMAL MISSING				
@ INCLUDES AUTOLYZED ANIMALS				
TUMOR SUMMARY				
TOTAL ANIMALS WITH PRIMARY TUMORS*	2	2	22	1
TOTAL PRIMARY TUMORS	2	2	24	1
TOTAL ANIMALS WITH BENIGN TUMORS	1	1		
TOTAL BENIGN TUMORS	1	1		
TOTAL ANIMALS WITH MALIGNANT TUMORS	1	1	22	1
TOTAL MALIGNANT TUMORS	1	1	24	1
TOTAL ANIMALS WITH SECONDARY TUMORS#	1		3	
TOTAL SECONDARY TUMORS	1		6	
TOTAL ANIMALS WITH TUMORS UNCERTAIN- BENIGN OR MALIGNANT				
TOTAL UNCERTAIN TUMORS				
TOTAL ANIMALS WITH TUMORS UNCERTAIN- PRIMARY OR METASTATIC				
TOTAL UNCERTAIN TUMORS				

* PRIMARY TUMORS: ALL TUMORS EXCEPT SECONDARY TUMORS
SECONDARY TUMORS: METASTATIC TUMORS OR TUMORS INVASIVE INTO AN ADJACENT ORGAN

APPENDIX C

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN RATS GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

TABLE C1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS NECROPSIED	14	15	32	33
ANIMALS EXAMINED HISTOPATHOLOGICALLY	14	15	32	33
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#TRACHEA	(12)	(15)	(28)	(33)
INFLAMMATION, ACUTE/CHRONIC		1 (7%)	4 (14%)	
#LUNG/BRONCHIOLE	(14)	(13)	(32)	(32)
HYPERPLASIA, LYMPHOID			1 (3%)	
#LUNG	(14)	(13)	(32)	(32)
INFLAMMATION, INTERSTITIAL		1 (8%)	4 (13%)	1 (3%)
BRONCHOPNEUMONIA SUPPURATIVE	3 (21%)		5 (16%)	
BRONCHOPNEUMONIA, CHRONIC			2 (6%)	
BRONCHOPNEUMONIA CHRONIC SUPPURA			4 (13%)	1 (3%)
HYPERPLASIA, LYMPHOID		1 (8%)	2 (6%)	
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(13)	(15)	(30)	(33)
ATROPHY, NOS		2 (13%)	5 (17%)	26 (79%)
#SPLEEN	(14)	(15)	(31)	(32)
INFLAMMATION, NECROTIZING				1 (3%)
NECROSIS, NOS				3 (9%)
#LYMPH NODE	(7)	(13)	(22)	(28)
INFLAMMATION, NECROTIZING				1 (4%)
CIRCULATORY SYSTEM				
#HEART/ATRIUM	(14)	(13)	(32)	(32)
THROMBOSIS, NOS				1 (3%)

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
#MYOCARDIUM	(14)	(13)	(32)	(32)
INFLAMMATION, NECROTIZING				1 (3%)
DEGENERATION, NOS				1 (3%)
DIGESTIVE SYSTEM				
#LIVER	(14)	(14)	(31)	(33)
CONGESTION, PASSIVE				1 (3%)
CONGESTION, CHRONIC PASSIVE				1 (3%)
INFLAMMATION, NECROTIZING				1 (3%)
NECROSIS, COAGULATIVE				2 (6%)
NECROSIS, CENTRAL			5 (16%)	3 (9%)
#LIVER/CENTRILOBULAR	(14)	(14)	(31)	(33)
NECROSIS, NOS				1 (3%)
NECROSIS, COAGULATIVE				8 (24%)
*BILE DUCT	(14)	(15)	(32)	(33)
INFLAMMATION, CHRONIC			1 (3%)	
#GASTRIC SUBMUCOSA	(14)	(15)	(31)	(33)
ABSCESS, CHRONIC				1 (3%)
URINARY SYSTEM				
#KIDNEY	(14)	(15)	(31)	(32)
HYDRONEPHROSIS	1 (7%)			
INFLAMMATION, INTERSTITIAL			2 (6%)	
INFLAMMATION, SUPPURATIVE				1 (3%)
GLOMERULONEPHRITIS, MEMBRANOUS	3 (21%)	1 (7%)	1 (3%)	
PYELONEPHRITIS, ACUTE/CHRONIC				1 (3%)
INFLAMMATION, CHRONIC			1 (3%)	
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
#PROSTATE	(14)	(15)	(28)	(30)
INFLAMMATION, SUPPURATIVE				1 (3%)
NERVOUS SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C1. MALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	7	12	4	4
AUTOLYSIS/NO NECROPSY	1		3	2

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE RATS GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	15	35	35
ANIMALS NECROPSIED	15	15	31	31
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	15	31	31
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#TRACHEA	(14)	(15)	(29)	(28)
INFLAMMATION, ACUTE/CHRONIC			1 (3%)	
#LUNG	(15)	(14)	(30)	(30)
INFLAMMATION, INTERSTITIAL		1 (7%)	1 (3%)	2 (7%)
BRONCHOPNEUMONIA SUPPURATIVE	1 (7%)		4 (13%)	
BRONCHOPNEUMONIA NECROTIZING				1 (3%)
BRONCHOPNEUMONIA CHRONIC SUPPURA	1 (7%)			
HYPERPLASIA, LYMPHOID		1 (7%)		1 (3%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(15)	(15)	(30)	(29)
ATROPHY, NOS			2 (7%)	15 (52%)
CIRCULATORY SYSTEM				
NONE				
DIGESTIVE SYSTEM				
#LIVER/CENTRILOBULAR	(14)	(14)	(31)	(31)
NECROSIS, NOS			1 (3%)	
NECROSIS, COAGULATIVE				4 (13%)
CYTOLOGIC DEGENERATION				1 (3%)
URINARY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
*MAMMARY GLAND	(15)	(15)	(31)	(31)
HYPERPLASIA, CYSTIC	1 (7%)			
#UTERUS/ENDOMETRIUM	(15)	(15)	(30)	(29)
INFLAMMATION, SUPPURATIVE	1 (7%)	2 (13%)	3 (10%)	
INFLAMMATION, CHRONIC SUPPURATIV			3 (10%)	
#OVARY	(10)	(13)	(23)	(27)
INFLAMMATION, SUPPURATIVE			1 (4%)	
INFLAMMATION, CHRONIC SUPPURATIV			3 (13%)	1 (4%)
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
NONE				
ALL OTHER SYSTEMS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE C2. FEMALE RATS: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	4	7	9	10
AUTO/NECROPSY/HISTO PERF				2
AUTOLYSIS/NO NECROPSY			4	4

\# NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX D

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MICE GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

TABLE D1.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN MALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	16	35	35
ANIMALS NECROPSIED	15	13	31	32
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	13	30	30
INTEGUMENTARY SYSTEM				
*SKIN	(15)	(13)	(31)	(32)
EPIDERMAL INCLUSION CYST	1 (7%)			
RESPIRATORY SYSTEM				
#LUNG	(15)	(13)	(29)	(30)
INFLAMMATION, INTERSTITIAL		1 (8%)	4 (14%)	
BRONCHOPNEUMONIA SUPPURATIVE			1 (3%)	
PERIVASCULITIS			1 (3%)	
HYPERPLASIA, ALVEOLAR EPITHELIUM				1 (3%)
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(15)	(13)	(29)	(25)
ATROPHY, NOS		1 (8%)	3 (10%)	
#SPLEEN	(15)	(13)	(26)	(28)
HYPERPLASIA, HEMATOPOIETIC			2 (8%)	
HYPERPLASIA, RETICULUM CELL			1 (4%)	1 (4%)
HEMATOPOIESIS		1 (8%)		
#MESENTERIC L. NODE	(15)	(3)	(23)	(20)
INFLAMMATION, SUPPURATIVE			1 (4%)	
INFLAMMATION, HEMORRHAGIC			1 (4%)	
CIRCULATORY SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
DIGESTIVE SYSTEM				
#LIVER	(15)	(13)	(30)	(30)
PERIVASCULITIS			1 (3%)	
NECROSIS, NOS			1 (3%)	
NECROSIS, COAGULATIVE			1 (3%)	
NECROSIS, CENTRAL			1 (3%)	
HYPERPLASIA, NODULAR	2 (13%)			
HYPERPLASIA, HEMATOPOIETIC			1 (3%)	
URINARY SYSTEM				
#KIDNEY	(15)	(13)	(28)	(29)
PYELONGPHRITIS, ACUTE/CHRONIC			1 (4%)	
INFLAMMATION, CHRONIC		1 (8%)		
#U. BLADDER/SUBMUCOSA	(14)	(12)	(26)	(21)
HEMORRHAGE		1 (8%)		
ENDOCRINE SYSTEM				
NONE				
REPRODUCTIVE SYSTEM				
*SEMINAL VESICLE	(15)	(13)	(31)	(32)
INFLAMMATION, SUPPURATIVE		1 (8%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				
MUSCULOSKELETAL SYSTEM				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D1. MALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
BODY CAVITIES				
*PERITONEUM	(15)	(13)	(31)	(32)
INFLAMMATION, CHRONIC		1 (8%)		1 (3%)
*MESENTERY	(15)	(13)	(31)	(32)
NECROSIS, FAT				1 (3%)
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	10	9	13	22
AUTO/NECROPSY/NO HISTO			1	2
AUTOLYSIS/NO NECROPSY		3	4	3

@ NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2.

SUMMARY OF THE INCIDENCE OF NONNEOPLASTIC LESIONS IN FEMALE MICE GIVEN INTRAPERITONEAL INJECTIONS OF 5-AZACYTIDINE

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
ANIMALS INITIALLY IN STUDY	15	14	35	35
ANIMALS NECROPSIED	15	14	29	30
ANIMALS EXAMINED HISTOPATHOLOGICALLY	15	13	29	30
INTEGUMENTARY SYSTEM				
NONE				
RESPIRATORY SYSTEM				
#LUNG	(15)	(13)	(28)	(30)
INFLAMMATION, INTERSTITIAL	2 (13%)	1 (8%)	1 (4%)	
HYPERPLASIA, LYMPHOID	3 (20%)		2 (7%)	
HEMATOPOIETIC SYSTEM				
#BONE MARROW	(13)	(13)	(28)	(29)
ATROPHY, NOS				10 (34%)
#SPLEEN	(12)	(13)	(29)	(29)
HYPERPLASIA, HEMATOPOIETIC		1 (8%)	1 (3%)	1 (3%)
HYPERPLASIA, RETICULUM CELL			1 (3%)	
HYPERPLASIA, LYMPHOID			2 (7%)	
#MESENTERIC L. NODE	(10)	(9)	(24)	(23)
HYPERPLASIA, RETICULUM CELL			1 (4%)	
#THYMUS	(7)	(8)	(10)	
HYPERPLASIA, HEMATOPOIETIC			1 (10%)	
CIRCULATORY SYSTEM				
#HEART	(14)	(12)	(28)	(30)
PERIARTERITIS	1 (7%)			
DIGESTIVE SYSTEM				
#LIVER	(15)	(13)	(29)	(30)
NECROSIS, FOCAL				1 (3%)
NECROSIS, COAGULATIVE				1 (3%)
HYPERPLASIA, FOCAL			1 (3%)	

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
*BILE DUCT	(15)	(14)	(29)	(30)
INFLAMMATION, ACUTE/CHRONIC	1 (7%)			
#PANCREAS	(13)	(13)	(25)	(27)
INFLAMMATION, CHRONIC				1 (4%)
NECROSIS, FOCAL				1 (4%)
URINARY SYSTEM				
#KIDNEY	(15)	(13)	(29)	(29)
AMYLOIDOSIS				1 (3%)
#KIDNEY/GLOMERULUS	(15)	(13)	(29)	(29)
NECROSIS, FOCAL				1 (3%)
ENDOCRINE SYSTEM				
#THYROID	(13)	(9)	(13)	(21)
NECROSIS, FOCAL				1 (5%)
#PARATHYROID	(4)	(2)	(5)	(7)
PERIARTERITIS	1 (25%)			
REPRODUCTIVE SYSTEM				
#UTERUS	(15)	(13)	(28)	(27)
HYPERPLASIA, RETICULUM CELL			1 (4%)	
#UTERUS/ENDOMETRIUM	(15)	(13)	(28)	(27)
INFLAMMATION, NECROTIZING		1 (8%)		
HYPERPLASIA, CYSTIC	1 (7%)			
#UTERUS/MYOMETRIUM	(15)	(13)	(28)	(27)
INFLAMMATION, NECROTIZING		1 (8%)		
NERVOUS SYSTEM				
NONE				
SPECIAL SENSE ORGANS				
NONE				

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

TABLE D2. FEMALE MICE: NONNEOPLASTIC LESIONS (CONTINUED)

	UNTREATED CONTROL	VEHICLE CONTROL	LOW DOSE	HIGH DOSE
MUSCULOSKELETAL SYSTEM				
NONE				
BODY CAVITIES				
*PERITONEUM	(15)	(14)	(29)	(30)
INFLAMMATION, CHRONIC				1 (3%)
ALL OTHER SYSTEMS				
NONE				
SPECIAL MORPHOLOGY SUMMARY				
NO LESION REPORTED	7	9	5	15
AUTO/NECROPSY/NO HISTO		1		
AUTOLYSIS/NO NECROPSY			6	5

NUMBER OF ANIMALS WITH TISSUE EXAMINED MICROSCOPICALLY
* NUMBER OF ANIMALS NECROPSIED

APPENDIX E

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN RATS TREATED WITH 5-AZACYTIDINE

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Treated with 5-Azacytidine[a]

Topography: Morphology	Vehicle Control	Low Dose
Hematopoietic System: Lymphoma, Granulocytic Leukemia, or Sarcoma[b]	0/15 (0)	4/32 (13)
P Values[c,d]	--	N.S.
Relative Risk (Vehicle Control)[e]		Infinite
Lower Limit		0.463
Upper Limit		Infinite
Weeks to First Observed Tumor	--	73
Testis: Interstitial-cell Tumor[b]	0/15 (0)	2/28 (7)
P Values[c,d]	--	N.S.
Relative Risk (Vehicle Control)[e]		Infinite
Lower Limit		0.168
Upper Limit		Infinite
Weeks to First observed Tumor	--	80

[a]Treated groups received doses of 2.6 or 5.2 mg/kg by injection three times per week for 34 weeks.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

Table E1. Analyses of the Incidence of Primary Tumors in Male Rats Treated with 5-Azacytidine[e]

(continued)

[c]Beneath the incidence of tumors in the treated group is the probability level for the Fisher exact test for the comparison of that group with the vehicle-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in the treated group than in the control group.

[e]The 95% confidence interval of the relative risk between the treated group and the control group.

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Treated with 5-Azacytidine[a]

Topography: Morphology	Vehicle Control	Low Dose
Pituitary: Chromophobe Adenoma[b]	4/15 (27)	0/30 (0)
P Values[c,d]	--	P = 0.009(N)
Relative Risk (Vehicle Control)[e]		0.000
Lower Limit		0.000
Upper Limit		0.521
Weeks to First Observed Tumor	65	--
Mammary Gland: Papillary Adenocarcinoma, Fibroadenoma, or Adenocarcinoma, NOS (not otherwise specified)[b]	3/15 (20)	13/31 (42)
P Values[c,d]	--	N.S.
Relative Risk (Vehicle Control)[e]		2.097
Lower Limit		0.716
Upper Limit		10.013
Weeks to First Observed Tumor	76	26

[a]Treated groups received doses of 2.6 or 5.2 mg/kg by injection three times per week for 34 weeks.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

Table E2. Analyses of the Incidence of Primary Tumors in Female Rats Treated with 5-Azacytidine[a]

(continued)

[c]Beneath the incidence of tumors in the treated group is the probability level for the Fisher exact test for the comparison of that group with the vehicle-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]A negative trend (N) indicates a lower incidence in the treated group than in the control group.

[e]The 95% confidence interval of the relative risk between each treated group and the control group.

APPENDIX F

ANALYSES OF THE INCIDENCE OF PRIMARY TUMORS IN MICE TREATED WITH 5-AZACYTIDINE

Table F1. Analyses of the Incidence of Primary Tumors in Male Mice Treated with 5-Azacytidine[a]

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Lymphoma[b]	0/13 (0)	4/31 (13)	3/32 (9)
P Values[c,d]	--	N.S.	N.S.
Relative Risk (Vehicle Control)[e]		Infinite	Infinite
Lower Limit		0.421	0.265
Upper Limit		Infinite	Infinite
Weeks to First Observed Tumor	--	73	74

[a]Treated groups received doses of 2.2 or 4.4 mg/kg by injection three times per week for 52 weeks.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the vehicle-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]Since survival in the high-dose group was short, the tests for dose-related trend are not reported.

[e]The 95% confidence interval of the relative risk between each treated group and the control group.

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Treated with 5-Azacytidine[a]

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Multiple Sites:			
Lymphoma[b]	0/14 (0)	7/29 (24)	0/30 (0)
P Values[c,d]	--	P = 0.048	N.S.
Relative Risk (Vehicle Control)[e]		Infinite	--
Lower Limit		1.010	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	43	--
Hematopoietic System:			
Granulocytic Leukemia or Sarcoma[b]	0/14 (0)	10/29 (34)	0/30 (0)
P Values[c,d]	--	P = 0.010	N.S.
Relative Risk (Vehicle Control)[e]		Infinite	--
Lower Limit		1.554	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	53	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Treated with 5-Azacytidine[a]

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Hematopoietic System: Lymphoma, Granulocytic Leukemia, or Sarcoma[b]	0/14 (0)	17/29 (59)	0/30 (0)
P Values[c,d]	--	P < 0.001	N.S.
Relative Risk (Vehicle Control)[e]		Infinite	--
Lower Limit		2.848	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	43	--
Mammary Gland: Adenocarcinoma[b]	0/14 (0)	2/29 (7)	0/30 (0)
P Values[c,d]	--	N.S.	N.S.
Relative Risk (Vehicle Control)[e]		Infinite	--
Lower Limit		0.153	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	61	--

Table F2. Analyses of the Incidence of Primary Tumors in Female Mice Treated with 5-Azacytidine[a]

(continued)

Topography: Morphology	Vehicle Control	Low Dose	High Dose
Uterus: Sarcoma, NOS, or Leiomyosarcoma[b]	0/13 (0)	3/28 (11)	0/27 (0)
P Values[c,d]	--	N.S.	N.S.
Relative Risk (Vehicle Control)[e]		Infinite	--
Lower Limit		0.300	--
Upper Limit		Infinite	--
Weeks to First Observed Tumor	--	81	--

[a]Treated groups received doses of 2.2 or 4.4 mg/kg by injection three times per week for 52 weeks.

[b]Number of tumor-bearing animals/number of animals examined at site (percent).

[c]Beneath the incidence of tumors in a treated group is the probability level for the Fisher exact test for the comparison of that treated group with the vehicle-control group when $P < 0.05$; otherwise, not significant (N.S.) is indicated.

[d]Since survival in the high-dose group was short, the tests for dose-related trend are not reported.

[e]The 95% confidence interval of the relative risk between each treated group and the control group.

Review of the Bioassay of 5-Azacytidine*for Carcinogenicity by the Data Evaluation/Risk Assessment Subgroup of the Clearinghouse on Environmental Carcinogens

November 28, 1977

The Clearinghouse on Environmental Carcinogens was established in May, 1976 under the authority of the National Cancer Act of 1971 (P.L. 92-218). The purpose of the Clearinghouse is to advise on the National Cancer Institute's bioassay program to identify and evaluate chemical carcinogens in the environment to which humans may be exposed. The members of the Clearinghouse have been drawn from academia, industry, organized labor, public interest groups, State health officials, and quasi-public health and research organizations. Members have been selected on the basis of their experience in carcinogenesis or related fields and, collectively, provide expertise in organic chemistry, biochemistry, biostatistics, toxicology, pathology, and epidemiology. Representatives of various Governmental agencies participate as ad hoc members. The Data Evaluation/Risk Assessment Subgroup of the Clearinghouse is charged with the responsibility of providing a peer review of NCI bioassay reports on chemicals studied for carcinogenicity. In this context, below is the edited excerpt from the minutes of the Subgroup's meeting at which 5-Azacytidine was reviewed.

The primary reviewer commented that the severe toxicity of the drug resulted in so many deaths that meaningful statistical analyses could not be done on the treated rats or male mice. In female mice, tumors of the hematopoietic system were associated with treatment. Bone marrow atrophy was a significant finding in the treated rats. The reviewer agreed with the staff's conclusion that 5-Azacytidine induced lymphomas and granulocytic leukemias in the treated female mice. The secondary reviewer also agreed with the conclusion given in the report. A discussion ensued as to the possible mechanism by which 5-Azacytidine exerts its effect.

Despite the inadequacies of the study, a motion was made that there was sufficient evidence to conclude that 5-Azacytidine induced hematopoietic neoplasms. The motion was seconded and approved by all present except Mr. Garfinkel, who opposed it based on the small animal groups and high early mortality.

Members present were:

Gerald N. Wogan (Chairman), Massachusetts Institute of Technology
Lawrence Garfinkel, American Cancer Society
Henry C. Pitot, University of Wisconsin Medical Center
George Roush, Jr., Monsanto Company
Verald K. Rowe, Dow Chemical U.S.A.
Michael B. Shimkin, University of California at San Diego
Louise Strong, University of Texas Health Sciences Center
John H. Weisburger, American Health Foundation

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* Subsequent to this review, changes may have been made in the bioassay report either as a result of the review or other reasons. Thus, certain comments and criticisms reflected in the review may no longer be appropriate.

*U S GOVERNMENT PRINTING OFFICE 1978 260-899/3017 1-3

DHEW Publication No. (NIH) 78-842

Attachment A-8

HIGHLIGHTS OF PRESCRIBING INFORMATION
These highlights do not include all the information needed to use ONUREG safely and effectively. See full prescribing information for ONUREG.

ONUREG (azacitidine) tablets, for oral use
Initial U.S. Approval: 2004

INDICATIONS AND USAGE

ONUREG is a nucleoside metabolic inhibitor indicated for continued treatment of adult patients with acute myeloid leukemia who achieved first complete remission (CR) or complete remission with incomplete blood count recovery (CRi) following intensive induction chemotherapy and are not able to complete intensive curative therapy (1).

DOSAGE AND ADMINISTRATION

- **Do not substitute ONUREG for intravenous or subcutaneous azacitidine. The indications and dosing regimen for ONUREG differ from that of intravenous or subcutaneous azacitidine (2.1, 5.1).**
- Administer ONUREG 300 mg orally once daily on Days 1 through 14 of each 28-day cycle (2.2).
- Administer an antiemetic before each dose for at least the first 2 cycles (2.2).

DOSAGE FORMS AND STRENGTHS

Tablets: 200 mg and 300 mg (3).

CONTRAINDICATIONS

History of severe hypersensitivity to azacitidine or its components (4).

WARNINGS AND PRECAUTIONS

- Risks of Substitution with Other Azacitidine Products: Do not substitute ONUREG for intravenous or subcutaneous azacitidine (2.1, 5.1).
- Myelosuppression: Monitor complete blood counts every other week for the first 2 cycles and prior to the start of each cycle thereafter. Increase monitoring to every other week for the 2 cycles after any dose reduction. Withhold and then resume at same or reduced dose or discontinue ONUREG based on severity (2.3, 5.2).
- Embryo-Fetal Toxicity: Can cause fetal harm. Advise patients of the potential risk to a fetus and use of effective contraception (5.4, 8.1, 8.3).

ADVERSE REACTIONS

The most common adverse reactions (≥ 10%) are nausea, vomiting, diarrhea, fatigue/asthenia, constipation, pneumonia, abdominal pain, arthralgia, decreased appetite, febrile neutropenia, dizziness, and pain in extremity.

To report SUSPECTED ADVERSE REACTIONS, contact Celgene Corporation at 1-888-423-5436 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.

USE IN SPECIFIC POPULATIONS

Lactation: Advise not to breastfeed (8.2).

See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling

Revised: 9/2020

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FULL PRESCRIBING INFORMATION

1 INDICATIONS AND USAGE

ONUREG is indicated for continued treatment of adult patients with acute myeloid leukemia who achieved first complete remission (CR) or complete remission with incomplete blood count recovery (CRi) following intensive induction chemotherapy and are not able to complete intensive curative therapy.

2 DOSAGE AND ADMINISTRATION

2.1 Important Administration Information

Do not substitute ONUREG for intravenous or subcutaneous azacitidine. The indications and dosing regimen for ONUREG differ from that of intravenous or subcutaneous azacitidine *[see Warnings and Precautions (5.1)].*

2.2 Recommended Dosage

The recommended dosage of ONUREG is 300 mg orally once daily with or without food on Days 1 through 14 of each 28-day cycle. Continue ONUREG until disease progression or unacceptable toxicity.

Administer an antiemetic 30 minutes prior to each dose of ONUREG for the first 2 cycles. Antiemetic prophylaxis may be omitted after 2 cycles if there has been no nausea and vomiting.

If the absolute neutrophil count (ANC) is less than 0.5 Gi/L on Day 1 of a cycle, do not administer ONUREG. Delay the start of the cycle until the ANC is 0.5 Gi/L or more.

Instruct patients on the following:

- Do not split, crush, or chew ONUREG tablets.
- Take a dose about the same time each day.
- If a dose of ONUREG is missed, or not taken at the usual time, take the dose as soon as possible on the same day, and resume the normal schedule the following day. Do not take 2 doses on the same day.
- If a dose is vomited, do not take another dose on the same day. Resume the normal schedule the following day.

ONUREG is a hazardous drug. Follow applicable special handling and disposal procedures.[1]

2.3 Monitoring and Dosage Modifications for Adverse Reactions

Monitor complete blood count every other week for the first 2 cycles and prior to the start of each cycle thereafter. Increase monitoring to every other week for the 2 cycles after any dose reduction for myelosuppression.

The recommended dosage modifications for adverse reactions are provided in Table 1.

Table 1: Recommended Dosage Modifications for Adverse Reactions

Adverse Reaction	Severity	Recommended Dosage Modification
Myelosuppression *[see Warnings and Precautions (5.2)]*	Neutrophils less than 0.5 Gi/L on Cycle Day 1	• Interrupt treatment. Resume at the same dose once neutrophils return to 0.5 Gi/L or higher.
	Neutrophils less than 1 Gi/L with fever at anytime	First Occurrence • Interrupt treatment. Resume at the same dose once neutrophils return to 1 Gi/L or higher. Occurrence in 2 Consecutive Cycles • Interrupt treatment. After neutrophils return to 1 Gi/L or higher, resume at reduced dose of 200 mg. • If a patient continues to experience febrile neutropenia after dose reduction, reduce the treatment duration by 7 days. • If febrile neutropenia reoccurs after dose and schedule reduction, discontinue ONUREG.
	Platelets less than 50 Gi/L with bleeding	First Occurrence • Interrupt dose. Resume at the same dose once platelets return to 50 Gi/L or higher. Occurrence in 2 Consecutive Cycles • Interrupt dose. After platelets return to 50 Gi/L or higher, resume at reduced dose of 200 mg. • If a patient continues to experience thrombocytopenia with bleeding after dose reduction, reduce the treatment duration by 7 days. • If thrombocytopenia with bleeding reoccurs after dose and schedule reduction, discontinue ONUREG.

Adverse Reaction	Severity	Recommended Dosage Modification
Gastrointestinal Toxicity *[see Adverse Reactions (6.1)]*	Grade 3 or 4 Nausea or Vomiting	• Interrupt dose. Resume at the same dose once toxicity has resolved to Grade 1 or lower. • If toxicity reoccurs, interrupt dose until resolved to Grade 1 or lower. Resume at reduced dose of 200 mg. • If a patient continues to experience the toxicity after dose reduction, reduce the treatment duration by 7 days. • If the toxicity continues or reoccurs after dose and schedule reduction, discontinue ONUREG.
	Grade 3 or 4 Diarrhea	• Interrupt dose. Resume at the same dose once toxicity has resolved to Grade 1 or lower. • If toxicity reoccurs, interrupt dose until resolved to Grade 1 or lower. Resume at reduced dose of 200 mg. • If a patient continues to experience the toxicity after dose reduction, reduce the treatment duration by 7 days. • If the toxicity continues or reoccurs after dose and schedule reduction, discontinue ONUREG.
Other Adverse Reactions *[see Adverse Reactions (6.1)]*	Grade 3 or 4	• Interrupt dose and provide medical support. Resume at the same dose once toxicity has resolved to Grade 1 or lower. • If toxicity re-occurs, interrupt dose until resolved to Grade 1 or lower. Resume at reduced dose of 200 mg. • If a patient continues to experience the toxicity after dose reduction, reduce the treatment duration by 7 days. • If the toxicity continues or reoccurs after dose and schedule reduction, discontinue ONUREG.

3 DOSAGE FORMS AND STRENGTHS

Tablets:

- 200 mg, pink, oval, film-coated tablet with debossed “200” on one side and “ONU” on the other side.
- 300 mg, brown, oval, film-coated tablet with debossed “300” on one side and “ONU” on the other side.

4 CONTRAINDICATIONS

ONUREG is contraindicated in patients with known severe hypersensitivity to azacitidine or its components *[see Adverse Reactions (6.2), Description (11)]*.

5 WARNINGS AND PRECAUTIONS

5.1 Risks of Substitution with Other Azacitidine Products

Due to substantial differences in the pharmacokinetic parameters *[see Clinical Pharmacology (12.3)]*, the recommended dose and schedule for ONUREG are different from those for the intravenous or subcutaneous azacitidine products. Treatment of patients using intravenous or subcutaneous azacitidine at the recommended dosage of ONUREG may result in a fatal adverse reaction. Treatment of patients using ONUREG at the doses recommended for intravenous or subcutaneous azacitidine may not be effective.

Do not substitute ONUREG for intravenous or subcutaneous azacitidine *[see Dosage and Administration (2.1)]*.

5.2 Myelosuppression

New or worsening Grade 3 or 4 neutropenia and thrombocytopenia occurred in 49% and 22% of patients who received ONUREG, respectively. Febrile neutropenia occurred in 12%. A dose reduction was required for 7% and 2% of patients due to neutropenia and thrombocytopenia, respectively. Less than 1% of patients discontinued ONUREG due to either neutropenia or thrombocytopenia.

Monitor complete blood counts and modify the dosage as recommended *[see Dosage and Administration (2.2, 2.3)]*. Provide standard supportive care, including hematopoietic growth factors, if myelosuppression occurs.

5.3 Increased Early Mortality in Patients with Myelodysplastic Syndromes

In AZA-MDS-003 (NCT01566695), 216 patients with red blood cell transfusion-dependent anemia and thrombocytopenia due to myelodysplastic syndromes were randomized to ONUREG or placebo. One-hundred and seven patients received a median of 5 cycles of ONUREG 300 mg daily for 21 days of a 28-day cycle. Enrollment was discontinued early due to a higher incidence of early fatal and/or serious adverse reactions in patients who received ONUREG compared with placebo. The most frequent fatal adverse reaction was sepsis. The safety and effectiveness of ONUREG for treatment of myelodysplastic syndromes have not been established. Treatment of patients with myelodysplastic syndromes with ONUREG is not recommended outside of controlled trials.

5.4 Embryo-Fetal Toxicity

Based on the mechanism of action and findings in animals, ONUREG can cause fetal harm when administered to a pregnant woman. Azacitidine administered to pregnant rats via a single intraperitoneal dose less than the recommended human daily dose of oral azacitidine on a mg/m^2 basis caused fetal death and anomalies.

Advise pregnant women of the potential risk to a fetus. Advise females of reproductive potential to use effective contraception during treatment with ONUREG and for at least 6 months after the last dose. Advise males with female partners of reproductive potential to use effective contraception during treatment with ONUREG and for at least 3 months after the last dose *[see Use in Specific Populations (8.1, 8.3)]*.

6 ADVERSE REACTIONS

The following clinically significant adverse reactions are described elsewhere in the labeling:

- Myelosuppression *[see Warnings and Precautions (5.2)]*

6.1 Clinical Trials Experience

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice.

Acute Myeloid Leukemia

The safety of ONUREG was evaluated in QUAZAR *[see Clinical Studies (14)]*. Patients received ONUREG 300 mg (N=236) or placebo (N=233) orally once daily on Days 1 through 14 of each 28-day cycle. Among patients who received ONUREG, 71% were exposed for 6 months or longer, and 49% were exposed for greater than one year. The median duration of exposure to

ONUREG was 11.6 months (range: 0.5 to 74.3 months) and the median number of cycles was 12 (range: 1 to 82 cycles).

Serious adverse reactions occurred in 15% of patients who received ONUREG. Serious adverse reactions in ≥ 2% of patients who received ONUREG were pneumonia (8%) and febrile neutropenia (7%). One fatal adverse reaction (sepsis) occurred in a patient who received ONUREG.

Permanent discontinuation of ONUREG due to an adverse reaction occurred in 8% of patients. Adverse reactions which resulted in permanent discontinuation of ONUREG in > 1% of patients included nausea (2.1%), diarrhea (1.7%), and vomiting (1.3%). Interruptions of ONUREG due to an adverse reaction occurred in 35% of patients. Adverse reactions which required an interruption of ONUREG in > 5% of patients included neutropenia (20%), thrombocytopenia (8%), and nausea (6%).

Dose reductions of ONUREG due to an adverse reaction occurred in 14% of patients. Adverse reactions which required a dose reduction in > 1% of patients included neutropenia (6%), diarrhea (3.4%), thrombocytopenia (1.7%), and nausea (1.7%).

The most common (≥ 10%) adverse reactions were nausea, vomiting, diarrhea, fatigue/asthenia, constipation, pneumonia, abdominal pain, arthralgia, decreased appetite, febrile neutropenia, dizziness, and pain in extremity.

Table 2 summarizes the adverse reactions in QUAZAR.

Table 2: Adverse Reactions (≥ 5%) in Patients with AML Who Received ONUREG with a Difference Between Arms of > 2% Compared to Placebo in QUAZAR

Adverse Reaction	ONUREG (N=236)		Placebo (N=233)	
	All Grades (%)	Grade 3 or 4 (%)	All Grades (%)	Grade 3 or 4 (%)
Gastrointestinal disorders				
Nausea	65	3	24	< 1
Vomiting	60	3	10	0
Diarrhea	50	5	21	1
Constipation	39	1	24	0
Abdominal pain[a]	22	2	13	< 1
General disorders and administration site conditions				
Fatigue / asthenia[b]	44	4	25	1

Infections				
Pneumonia[c]	27	9	17	5
Musculoskeletal and connective tissue disorders				
Arthralgia	14	1	10	< 1
Pain in extremity	11	< 1	5	0
Metabolism and nutrition disorders				
Decreased appetite	13	1	6	1
Blood and lymphatic disorders				
Febrile neutropenia	12	11	8	8
Nervous system disorders				
Dizziness	11	0	9	0

[a] Grouped term includes abdominal pain, abdominal pain upper, abdominal discomfort, and gastrointestinal pain.
[b] Grouped term includes fatigue and asthenia.
[c] Broad scope term includes influenza, pneumonia, respiratory tract infection, respiratory tract infection viral, bronchopulmonary aspergillosis, lung infection, Staphylococcal infection, atypical pneumonia, lower respiratory tract infection, lung abscess, Pneumocystis jirovecii pneumonia, pneumonia bacterial, pneumonia fungal, Pseudomonas infection, hemoptysis, productive cough, pleural effusion, atelectasis, pleuritic pain, rales, Enterobacter test positive, and Hemophilus test positive.

Clinically relevant adverse reactions that did not meet criteria for inclusion in Table 2 were weight decreased (4%) in patients who received ONUREG.

Neutropenia, thrombocytopenia, and anemia of any grade occurred in 74%, 65%, and 25% of patients who received ONUREG. Table 3 summarizes select Grades 3 or 4 hematological laboratory abnormalities in QUAZAR.

Table 3: Selected Hematological Laboratory Abnormalities That Worsened from Baseline in Patients Who Received ONUREG in QUAZAR

Laboratory Abnormality	**ONUREG**		**Placebo**	
	Baseline Grade 0-2 N	**Post-Baseline Grade 3 or 4 n (%)**	**Baseline Grade 0-2 N**	**Post-Baseline Grade 3 or 4 n (%)**
Neutropenia	223	109 (49)	217	50 (23)
Thrombocytopenia	222	46 (21)	212	22 (10)
Anemia	229	10 (4)	223	7 (3)

6.2 Postmarketing Experience

The following adverse reactions have been identified during postapproval use of intravenous or subcutaneous azacitidine. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure.

- Hypersensitivity reaction
- Interstitial lung disease
- Tumor lysis syndrome
- Sweet's syndrome (acute febrile neutrophilic dermatosis)
- Necrotizing fasciitis (including fatal cases)
- Differentiation syndrome

8 USE IN SPECIFIC POPULATIONS

8.1 Pregnancy

Risk Summary

Based on its mechanism of action *[see Clinical Pharmacology (12.1)]* and findings in animals, ONUREG can cause fetal harm when administered to a pregnant woman. There are no available data on ONUREG use in pregnant women to evaluate for a drug-associated risk. Azacitidine was teratogenic and caused embryo-fetal lethality in animals at doses less than the recommended human daily dose of oral azacitidine on a mg/m^2 basis *(see Data)*. Advise pregnant women of the potential risk to the fetus.

The estimated background of major birth defects and miscarriage for the indicated population is unknown. All pregnancies have a background risk of birth defect, loss, or other adverse outcomes. In the U.S. general population, the estimated background risk of major birth defects and miscarriage in clinically recognized pregnancies is 2% to 4% and 15% to 20%, respectively.

Data

Animal Data

No reproductive or developmental toxicity studies have been conducted with oral azacitidine.

Early embryotoxicity studies in mice revealed a 44% frequency of intrauterine embryonal death (increased resorption) after a single intraperitoneal injection of 6 mg/m^2 azacitidine (at doses less than the recommended human daily dose of oral azacitidine on a mg/m^2 basis) on gestation

Day 10. Developmental abnormalities in the brain have been detected in mice given azacitidine on or before gestation Day 15 at doses of approximately 3 to 12 mg/m^2 (at doses less than the recommended human daily dose on a mg/m^2 basis).

In rats, azacitidine was clearly embryotoxic when given an intraperitoneal injection on gestation Days 4 to 8 (postimplantation) at a dose of 6 mg/m^2 (at doses less than the recommended human daily dose on a mg/m^2 basis), although treatment in the preimplantation period (on gestation Days 1 to 3) had no adverse effect on the embryos. Azacitidine caused multiple fetal abnormalities in rats after a single intraperitoneal dose of 3 to 12 mg/m^2 (at doses less than the recommended human daily dose on a mg/m^2 basis) given on gestation Days 9, 10, 11, or 12. In this study, azacitidine caused fetal death when administered at 3 to 12 mg/m^2 on gestation Days 9 and 10; average live animals per litter was reduced to 9% of control at the highest dose on gestation Day 9. Fetal anomalies included: CNS anomalies (exencephaly/encephalocele), limb anomalies (micromelia, club foot, syndactyly, oligodactyly), and others (micrognathia, gastroschisis, edema, and rib abnormalities).

8.2 Lactation

Risk Summary

There are no data regarding the presence of azacitidine in human milk or the effects on the breastfed child or milk production. Because of the potential for serious adverse reactions in the breastfed child, advise women not to breastfeed during treatment with ONUREG and for 1 week after the last dose.

8.3 Females and Males of Reproductive Potential

ONUREG can cause embryo-fetal harm when administered to pregnant women *[see Use in Specific Populations (8.1)]*.

Pregnancy Testing

Pregnancy testing is recommended for females of reproductive potential before starting ONUREG.

Contraception

Females

Advise females of reproductive potential to use effective contraception during treatment with ONUREG and for at least 6 months after the last dose.

Males

Advise males with female partners of reproductive potential to use effective contraception during treatment with ONUREG and for at least 3 months after the last dose.

Infertility

Based on animal data, ONUREG may impair male or female fertility *[see Nonclinical Toxicology (13.1)]*.

8.4 Pediatric Use

The safety and effectiveness of ONUREG in pediatric patients have not been established.

8.5 Geriatric Use

Of the 238 patients in QUAZAR who received ONUREG, 72% were 65 years of age or older, while 12% were 75 years of age or older. No overall differences in safety or effectiveness of ONUREG were observed between these patients and younger patients.

8.6 Renal Impairment

Monitor patients with severe renal impairment (creatinine clearance [CLcr] 15 to 29 mL/min calculated by Cockcroft-Gault formula) more frequently for adverse reactions and modify the ONUREG dosage for adverse reactions *[see Dosage and Administration (2.3)]*.

No dose adjustment of ONUREG is recommended for patients with mild to severe renal impairment (CLcr 15 to 89 mL/min) *[see Clinical Pharmacology (12.3)]*.

8.7 Hepatic Impairment

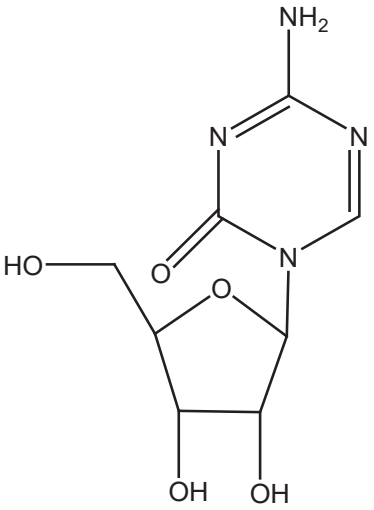
ONUREG has not been studied in patients with pre-existing severe hepatic impairment (total bilirubin > 3 × ULN).

A recommended dosage of ONUREG has not been established for patients with moderate hepatic impairment (total bilirubin > 1.5 to 3 × ULN).

No dose adjustment of ONUREG is recommended for patients with mild hepatic impairment (total bilirubin ≤ ULN and AST > ULN, or total bilirubin 1 to 1.5 × ULN and any AST) *[see Clinical Pharmacology (12.3)]*.

11 DESCRIPTION

Azacitidine is a nucleoside metabolic inhibitor with a molecular formula of $C_8H_{12}N_4O_5$ and a molecular weight of 244 g/mol. The chemical name is: 4-amino-1-β-D-ribofuranosyl-s-triazin-2(1H)-one and the chemical structural is:



Azacitidine is a white to off-white solid. Azacitidine was found to be soluble in aqueous media across a pH range from 1.0 to 7.0.

ONUREG (azacitidine) is supplied as film-coated tablets containing 200 mg or 300 mg of azacitidine for oral use. Each core tablet contains the following inactive ingredients: croscarmellose sodium, magnesium stearate, mannitol, and silicified microcrystalline cellulose. The 200 and 300 mg tablet coating contains hypromellose, lactose monohydrate, polyethylene glycol, titanium dioxide, and triacetin. In addition, the 200 mg tablet coating contains iron oxide red and the 300 mg tablet coating contains black iron oxide, iron oxide red, and iron oxide yellow.

12 CLINICAL PHARMACOLOGY

12.1 Mechanism of Action

Azacitidine is a pyrimidine nucleoside analog of cytidine that inhibits DNA/RNA methyltransferases. Azacitidine is incorporated into DNA and RNA following cellular uptake and enzymatic biotransformation to nucleotide triphosphates.

Incorporation of azacitidine into the DNA of cancer cells in vitro, including acute myeloid leukemia cells, inhibited DNA methyltransferases, reduced DNA methylation and altered gene expression, including re-expression of genes regulating tumor suppression and cell differentiation. Incorporation of azacitidine into the RNA of cancer cells, including leukemic cells, inhibited RNA methyltransferases, reduced RNA methylation, decreased RNA stability and decreased protein synthesis.

Antileukemic activity of azacitidine was demonstrated by reduction of cell viability and induction of apoptosis in AML cell lines in vitro. Azacitidine decreased tumor burden and increased survival in leukemic tumor models in vivo.

12.2 Pharmacodynamics

Greater reduction in global DNA methylation was observed with higher azacitidine plasma exposure in patients with AML administered ONUREG for 14 days of a 28-day cycle.

12.3 Pharmacokinetics

The systemic exposure of azacitidine is approximately dose proportional over the dose range of 120 mg to 600 mg once daily of ONUREG (0.4 to 2 times the recommended dosage). Following a single 300 mg dose of ONUREG, the mean (coefficient of variation [CV%]) C_{max} of azacitidine was 145 ng/mL (64%) and the mean AUC of azacitidine was 242 ng h/mL (65%). No accumulation was observed following ONUREG 300 mg once daily.

Absorption

The mean oral bioavailability is approximately 11% relative to subcutaneous administration. The median time to peak plasma concentration of azacitidine is 1 hour.

Effect of Food

A high-fat, high-calorie meal (approximately 800 to 1000 calories, 50% fat) did not affect $AUC_{0\text{-}INF}$ and decreased C_{max} by 21%.

Distribution

The mean (CV%) apparent volume of distribution (V_z/F) of azacitidine is 881 L (67%). The in vitro serum protein binding of azacitidine is approximately 6% to 12%. The blood-to-plasma ratio is approximately 0.3.

Elimination

The mean (CV%) terminal half-life is approximately 0.5 hours (27%) and the apparent clearance (CL/F) is 1240 L/hour (64%).

Metabolism

Azacitidine undergoes spontaneous hydrolysis and deamination mediated by cytidine deaminase.

Excretion

Following the administration of ONUREG 300 mg orally once daily, < 2% of the dose was recovered unchanged in the urine.

Specific Populations

Age (46 years to 93 years), sex, body weight (39.3 kg to 129 kg), mild hepatic impairment (total bilirubin ≤ ULN and AST > ULN, or total bilirubin 1 to 1.5 × ULN and any AST), and mild to moderate renal impairment (CLcr 30 to 89 mL/min) have no clinically meaningful effect on the pharmacokinetics of oral azacitidine. The effects of race/ethnicity, moderate to severe hepatic impairment (total bilirubin > 1.5 × ULN and any AST), and severe renal impairment (CLcr 15 to 29 mL/min) on the pharmacokinetics of oral azacitidine is unknown.

Severe renal impairment increased azacitidine exposure by approximately 70% after a single or 41% after multiple subcutaneous daily administration.

Drug Interaction Studies

Effect of Gastric Acid Reducing Agents on Azacitidine:

Coadministration of omeprazole (a proton pump inhibitor) with ONUREG increased azacitidine $AUC_{0\text{-}INF}$ by 19% and had no effect on C_{max}.

In Vitro Studies

Cytochrome P450 (CYP) Enzymes: Azacitidine does not inhibit CYP1A2, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4, or CYP2E1 at clinically relevant concentrations. Azacitidine is not an inducer of CYP1A2, CYP2C19, or CYP3A.

Transporter Systems: Azacitidine is not a substrate of P-glycoprotein (P-gp). Azacitidine does not inhibit P-gp, breast cancer resistance protein (BCRP), organic anion transporters (OAT) OAT1 and OAT3, organic anion transporting polypeptides (OATP) OATP1B1 and OATP1B3, or organic cation transporter (OCT) OCT2 at clinically relevant concentrations.

13 NONCLINICAL TOXICOLOGY

13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

The potential carcinogenicity of azacitidine was evaluated in mice and rats. Azacitidine induced tumors of the hematopoietic system in female mice at 2.2 mg/kg (6.6 mg/m^2, approximately 4% of the recommended human daily dose of oral azacitidine on a mg/m^2 basis) administered intraperitoneal 3 times per week for 52 weeks. An increased incidence of tumors in the lymphoreticular system, lung, mammary gland, and skin was seen in mice treated with intraperitoneal azacitidine at 2 mg/kg (6 mg/m^2, approximately 3% of the recommended human daily dose of oral azacitidine on a mg/m^2 basis) once a week for 50 weeks. A tumorigenicity study in rats dosed twice weekly at 15 or 60 mg/m^2 (approximately 8% to 32% of the recommended human daily dose of oral azacitidine on a mg/m^2 basis) revealed an increased incidence of testicular tumors compared with controls.

The mutagenic and clastogenic potential of azacitidine was tested in in vitro bacterial systems Salmonella typhimurium strains TA100 and several strains of trpE8, Escherichia coli strains WP14 Pro, WP3103P, WP3104P, and CC103; in an in vitro forward gene mutation assay in mouse lymphoma cells and human lymphoblast cells; and in an in vitro micronucleus assay in mouse L5178Y lymphoma cells and Syrian hamster embryo cells. Azacitidine was mutagenic in bacterial and mammalian cell systems. The clastogenic effect of azacitidine was shown by the induction of micronuclei in L5178Y mouse cells and Syrian hamster embryo cells.

Administration of azacitidine by intraperitoneal injection to male mice at 9.9 mg/m^2 (at doses less than the recommended human daily dose on a mg/m^2 basis) daily for 3 days prior to mating with untreated female mice resulted in decreased fertility and loss of offspring during subsequent embryonic and postnatal development. Treatment of male rats 3 times per week for 11 or 16 weeks at doses of 15 to 30 mg/m^2 (at doses less than the recommended human daily dose on a mg/m^2 basis) resulted in decreased weight of the testes and epididymides, decreased sperm counts accompanied by decreased pregnancy rates, and increased loss of embryos in mated females. In a related study, male rats treated for 16 weeks at 24 mg/m^2 resulted in an increase in abnormal embryos in mated females when examined on Day 2 of gestation.

14 CLINICAL STUDIES

The efficacy of ONUREG was evaluated in QUAZAR (NCT01757535), a multicenter, randomized, double-blind, placebo-controlled study. Eligible patients were ages 55 years or older, had AML, and were within 4 months of achieving first complete remission (CR) or complete remission with incomplete blood count recovery (CRi) with intensive induction chemotherapy. Patients may have received consolidation (see Table 4). Patients were excluded if they were candidates for hematopoietic stem cell transplantation at the time of screening.

A total of 472 patients who completed induction with or without consolidation therapy were randomized 1:1 to receive ONUREG 300 mg (n=238) or placebo (n=234) orally on Days 1 through 14 of each 28-day cycle. Randomization was stratified by age at time of induction therapy (55 to 64 vs. ≥ 65 years), cytogenetic risk category at time of induction therapy (intermediate risk vs. poor risk), prior history of MDS/CMML (yes vs. no), and received consolidation therapy following induction therapy (yes vs. no). Baseline demographic and disease characteristics are shown in Table 4.

Table 4: Baseline Demographics and Disease-Related Characteristics in QUAZAR

Parameter	**ONUREG (N=238)**	**Placebo (N=234)**
Age (years)		
Median (Min, Max)	68.0 (55, 86)	68.0 (55, 82)
Age Category, n (%)		

Parameter	ONUREG (N=238)	Placebo (N=234)
< 65 years	66 (28)	68 (29)
65 years to < 75 years	144 (61)	142 (61)
≥ 75 years	28 (12)	24 (10)
Sex, n (%)		
Male	118 (50)	127 (54)
Female	120 (50)	107 (46)
Race, n (%)		
White	216 (91)	197 (84)
Black or African American	2 (1)	6 (3)
Asian	6 (3)	20 (9)
Other	12 (5)	11 (5)
Not Collected or Reported	2 (1)	0 (0)
ECOG Performance Status, n (%)		
0	116 (49)	111 (47)
1	101 (42)	106 (45)
2	21 (9)	15 (6)
3	0 (0)	2 (1)
Cytogenetic Risk Status at Diagnosis, n (%)		
Intermediate Risk [1]	203 (85)	203 (87)
Poor Risk [2]	35 (15)	31 (13)
Initial AML Classification, n (%)		
AML with recurrent genetic abnormalities	39 (16)	46 (20)
AML with myelodysplasia-related changes	49 (21)	42 (18)
Therapy related myeloid neoplasms	2 (1)	0 (0)
AML not otherwise specified	148 (62)	145 (62)
Missing	0 (0)	1 (< 1)
Type of AML, n (%)		
Primary (de novo)	213 (89)	216 (92)
Secondary	25 (11)	18 (8)

Parameter	ONUREG (N=238)	Placebo (N=234)
Induction Response		
CR	187 (79)	197 (84)
CRi	51 (21)	37 (16)
Consolidation following induction therapy		
None	52 (22)	42 (18)
1 cycle	110 (46)	102 (44)
2 cycles	70 (29)	77 (33)
3 cycles	6 (3)	13 (6)
Disease status at study baseline		
CR	185 (78)	181 (77)
CRi	44 (18)	38 (16)
Not in CR or CRi	9 (4)	13 (6)
Not Reported	0	2 (1)

AML=Acute Myeloid Leukemia, MDS=Myelodysplastic Syndrome, CMML=Chronic Myelomonocytic Leukemia, ECOG=Eastern Cooperative Oncology Group, CR=Morphologic Complete Remission, CRi=Morphologic complete remission with incomplete blood count recovery.

[1] Intermediate risk was defined as normal cytogenetics +8, t(9;11), or Other undefined.

[2] Poor risk was defined as Complex (≥ 3 abnormalities): -5; 5q-; -7; 7q-; 11q23 - non t(9;11); inv(3); t(3;3); t(6;9); or t(9;22).

Source for Intermediate and Poor Risk: National Comprehensive Cancer Network Clinical Practice Guidelines in Oncology for Acute Myeloid Leukemia. National Comprehensive Cancer Network website. Available at http://www.nccn.org/professionals/physician_gls/PDF/aml.pdf. Accessed 01 Mar 2011.

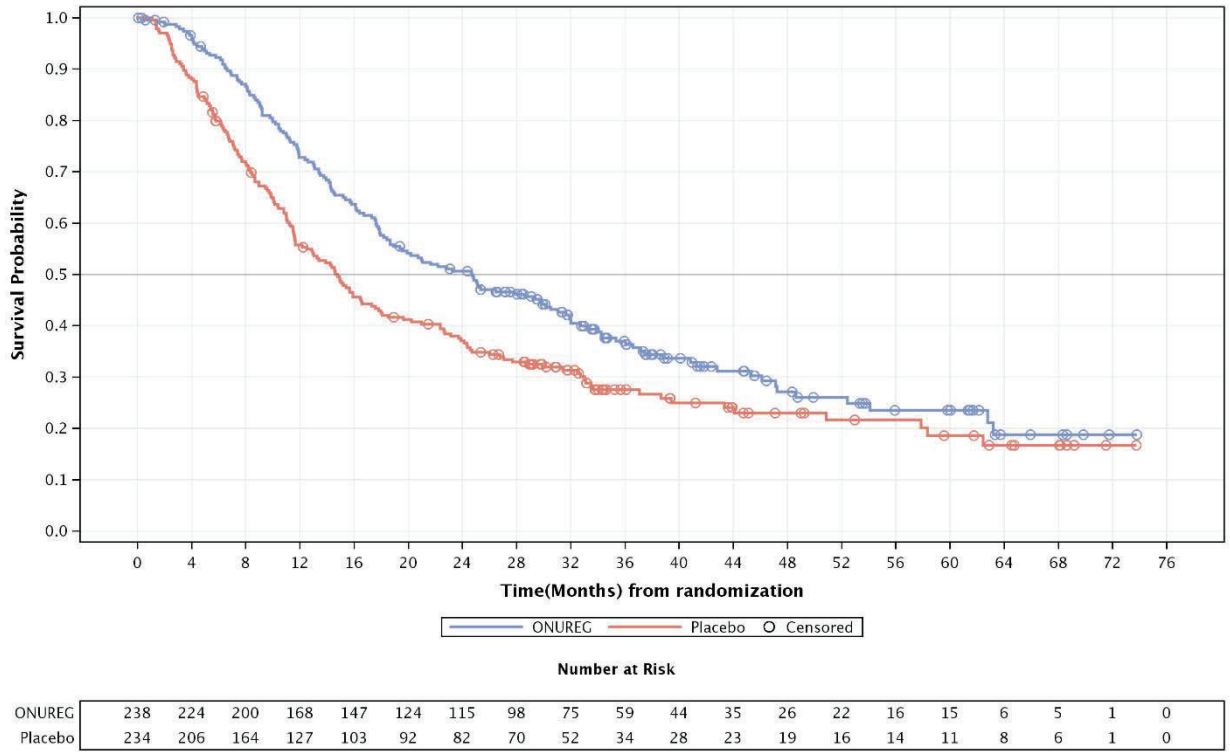
The efficacy of ONUREG was established on the basis of overall survival (OS). The trial demonstrated a statistically significant improvement in OS for patients randomized to ONUREG compared to placebo. A subgroup analysis showed consistency in the OS benefit for patients in either CR or CRi. The efficacy results are summarized in Table 5 and Figure 1.

Table 5: Efficacy Results (ITT Population) in QUAZAR

	ONUREG (N=238)	Placebo (N=234)
Overall Survival		
OS Events, n (%)	158 (66)	171 (73)
Median OS (95% CI) Months	24.7 (18.7, 30.5)	14.8 (11.7, 17.6)
Hazard Ratio (95% CI) [1]	0.69 (0.55, 0.86)	
p value [1]	0.0009	

[1] The hazard ratio is from a Cox proportional hazards model stratified by age (55 to 64 vs. ≥65 years), cytogenetic risk category at time of induction therapy (intermediate risk vs. poor risk), and received consolidation therapy (yes vs. no). The p-value is two-sided from a log-rank test stratified by the same factors.

Figure 1: Kaplan-Meier Curve for Overall Survival (ITT Population) in QUAZAR



15 REFERENCES

1. "OSHA Hazardous Drugs." *OSHA*. http://www.osha.gov/SLTC/hazardousdrugs/index.html

16 HOW SUPPLIED/STORAGE AND HANDLING

How Supplied

ONUREG tablets are available as:

- 200 mg: pink, oval, film-coated tablets with debossed "200" on one side and "ONU" on the other side.
- 300 mg: brown, oval, film-coated tablets with debossed "300" on one side and "ONU" on the other side.

Table 6 lists the package configurations and strengths.

Table 6: ONUREG Package Configurations and NDC Numbers

Package Configuration	Tablet Strength	NDC Number

Bottles of 14 with two desiccant cannisters	200 mg	59572-730-14
Bottles of 14 with two desiccant cannisters	300 mg	59572-740-14

Storage

Store bottles at 20ºC to 25ºC (68ºF to 77ºF); excursions permitted between 15ºC to 30º C (59ºF to 86ºF) [see USP Controlled Room Temperature].

Keep bottle tightly closed.

Store and dispense in the original bottle (with two desiccant canisters).

Handling and Disposal

ONUREG is a hazardous drug. Follow applicable special handling and disposal procedures.[1]

If powder comes in contact with skin, immediately and thoroughly wash with soap and water. If powder comes in contact with mucous membranes, immediately flush the area with water.

17 PATIENT COUNSELING INFORMATION

Advise the patient to read the FDA-approved patient labeling (Patient Information).

Myelosuppression

Advise patients of the risk of myelosuppression with ONUREG and of the need to monitor complete blood counts before and during treatment *[see Warnings and Precautions (5.2)]*.

Gastrointestinal Toxicity

Advise patients of the risk of gastrointestinal toxicity with ONUREG and of the potential need to use anti-emetic or anti-diarrheal medications during treatment *[see Adverse Reactions (6.1)]*.

Embryo-Fetal Toxicity

Advise pregnant women of the potential risk to a fetus. Advise females of reproductive potential to inform their healthcare provider of a known or suspected pregnancy *[see Warnings and Precautions (5.4), Use in Specific Populations (8.1)]*.

Advise females of reproductive potential to use effective contraception during treatment with ONUREG and for at least 6 months after the last dose *[see Use in Specific Populations (8.3)]*.

Advise males with female partners of reproductive potential to use effective contraception during treatment with ONUREG and for at least 3 months after the last dose *[see Use in Specific Populations (8.3)]*.

Lactation

Advise women not to breastfeed during treatment with ONUREG and for 1 week after the last dose *[see Use in Specific Populations (8.2)]*.

Administration

Advise patients to take ONUREG with or without food at about the same time each day and how to make up a missed or vomited dose. Advise patients to swallow tablets whole. Advise patients not to cut, split, crush, or chew the tablets *[see Dosage and Administration (2.2)]*.

Storage Instructions

Advise patients to keep ONUREG in the original container. Advise patients to keep the container tightly closed with both desiccant canisters inside and to not eat the desiccant canisters *[see How Supplied/Storage and Handling (16)]*.

Manufactured by: Celgene Corporation
A Wholly Owned Subsidiary of Bristol-Myers Squibb
86 Morris Avenue
Summit, NJ 07901

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ONUPI.001

Patient Information
ONUREG® (on-u-reg)
(azacitidine) tablets, for oral use

What is ONUREG?

ONUREG is a prescription medicine used for continued treatment of adults with acute myeloid leukemia (AML) who:

- had a first complete remission (CR) following intensive induction chemotherapy with or without recovery of your blood cell counts, **and**
- who are not able to complete intensive curative therapy.

It is not known if ONUREG is safe and effective in children under 18 years of age.

Do not take ONUREG if you:

- are allergic to azacitidine or any of the ingredients in ONUREG. See the end of this leaflet for a complete list of ingredients in ONUREG.

Before taking ONUREG, tell your healthcare provider about all of your medical conditions, including if you:

- have kidney or liver problems.
- are pregnant or plan to become pregnant. ONUREG can harm your unborn baby.
  Females who are able to become pregnant:
  - Your healthcare provider should perform a pregnancy test before you start treatment with ONUREG.
  - You should use effective birth control (contraception) during treatment and for at least 6 months after your last dose of ONUREG.
  - Tell your healthcare provider right away if you become pregnant during treatment with ONUREG.

  Males with a female sexual partner who can become pregnant:
  - You should use effective birth control (contraception) during treatment and for at least 3 months after your last dose of ONUREG.
- are breastfeeding or plan to breastfeed. It is not known if ONUREG passes into your breast milk. Do not breastfeed during treatment and for 1 week after your last dose of ONUREG.

Tell your healthcare provider about all the medicines you take**,** including prescription and over-the-counter medicines, vitamins, and herbal supplements.

How should I take ONUREG?

- Take ONUREG exactly as your healthcare provider tells you to take it.
- Your healthcare provider will prescribe an anti-nausea medicine for you to take to help prevent nausea and vomiting during your treatment with ONUREG.
  - Take the anti-nausea medicine 30 minutes before each dose of ONUREG.
  - Your healthcare provider may decide to stop the anti-nausea medicine after your second cycle of ONUREG, if you do not have any nausea or vomiting.
- Take ONUREG by mouth 1 time each day beginning on Day 1 through Day 14 of each 28-day cycle.
- Take ONUREG with or without food at about the same time each day.
- Swallow ONUREG tablets whole. Do not cut, split, crush, or chew the tablets.
- If the powder from ONUREG tablets comes in contact with your skin, wash the area well right away with soap and water.
- If the powder from ONUREG tablets comes in contact with your eyes or mouth (mucous membranes), flush the area right away with water.
- If you miss a dose of ONUREG, or if you do not take your dose at the usual time, take the dose as soon as possible that day. Take your next dose at the regular time the next day. Do not take 2 doses on the same day to make up for a missed dose.
- If you vomit after taking a dose of ONUREG, do not take another dose on the same day. Take your next dose at the regular time the next day.

What are the possible side effects of ONUREG?

ONUREG can cause serious side effects, including:

- **New or worsening low white blood cell counts (neutropenia). New or worsening low white blood cell counts are common but can also be severe during treatment with ONUREG. If your white blood cell counts become very low, you are at increased risk for infections.** Your healthcare provider will check your white blood cell counts before and during treatment with ONUREG. Your healthcare provider may prescribe a medicine to help increase your white blood cell count if needed.
  Tell your healthcare provider right away if you get any of the following symptoms:

- fever or chills
- body aches
- feeling very tired or weak
- unusual headaches

- **New or worsening low platelet counts (thrombocytopenia). Low platelet counts are common but can also be severe during treatment with ONUREG.** Your healthcare provider will check your platelet counts before and during treatment with ONUREG. Tell your healthcare provider right away if you have any unusual bruising or bleeding. Your healthcare provider may change your dose or tell you to stop taking ONUREG if you have low blood cell counts.

ONUREG may cause fertility problems in males and females, which may affect your ability to have children. Talk with your healthcare provider if you have concerns about fertility.

The most common side effects of ONUREG include:

- nausea and vomiting. See "How should I take ONUREG?"
- diarrhea. You may need to be treated with anti-diarrheal medicines.
- tiredness or weakness
- constipation
- stomach area (abdominal) pain
- pneumonia
- joint pain
- decreased appetite
- pain in arms or legs
- dizziness

These are not all of the possible side effects of ONUREG. Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

How should I store ONUREG?

- Store bottles of ONUREG tablets at room temperature between 68°F to 77°F (20°C to 25°C).
- Store ONUREG tablets in the original bottle.
- Bottles of ONUREG contain 2 drying agent (desiccant) canisters. **Do not eat the desiccant canisters.**
- Keep the ONUREG bottle tightly closed.
- Talk to your healthcare provider about how to safely throw away (dispose of) any unused or expired ONUREG.

Keep ONUREG and all medicines out of the reach of children

General information about the safe and effective use of ONUREG.

Medicines are sometimes prescribed for purposes other than those listed in a Patient Information leaflet. Do not use ONUREG for a condition for which it was not prescribed. Do not give ONUREG to other people, even if they have the same symptoms you have. It may harm them. You can ask your pharmacist or healthcare provider for information about ONUREG that is written for health professionals.

What are the ingredients in ONUREG?

Active ingredient: azacitidine

Inactive ingredients:

Each core tablet contains: croscarmellose sodium, magnesium stearate, mannitol, and silicified microcrystalline cellulose.

The pink 200 mg tablet coating contains: hypromellose, iron oxide red, lactose monohydrate, polyethylene glycol, titanium dioxide, and triacetin.

The brown 300 mg tablet coating contains: black iron oxide, hypromellose, iron oxide red, iron oxide yellow, lactose monohydrate, polyethylene glycol, titanium dioxide, and triacetin.

Manufactured by: Celgene Corporation, A Wholly Owned Subsidiary of Bristol Myers Squibb, 86 Morris Avenue, Summit, NJ 07901
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For more information, go to www.ONUREG.com or call 1-888-423-5436.

This Patient Information has been approved by the U.S. Food and Drug Administration. Issued: Sep 2020

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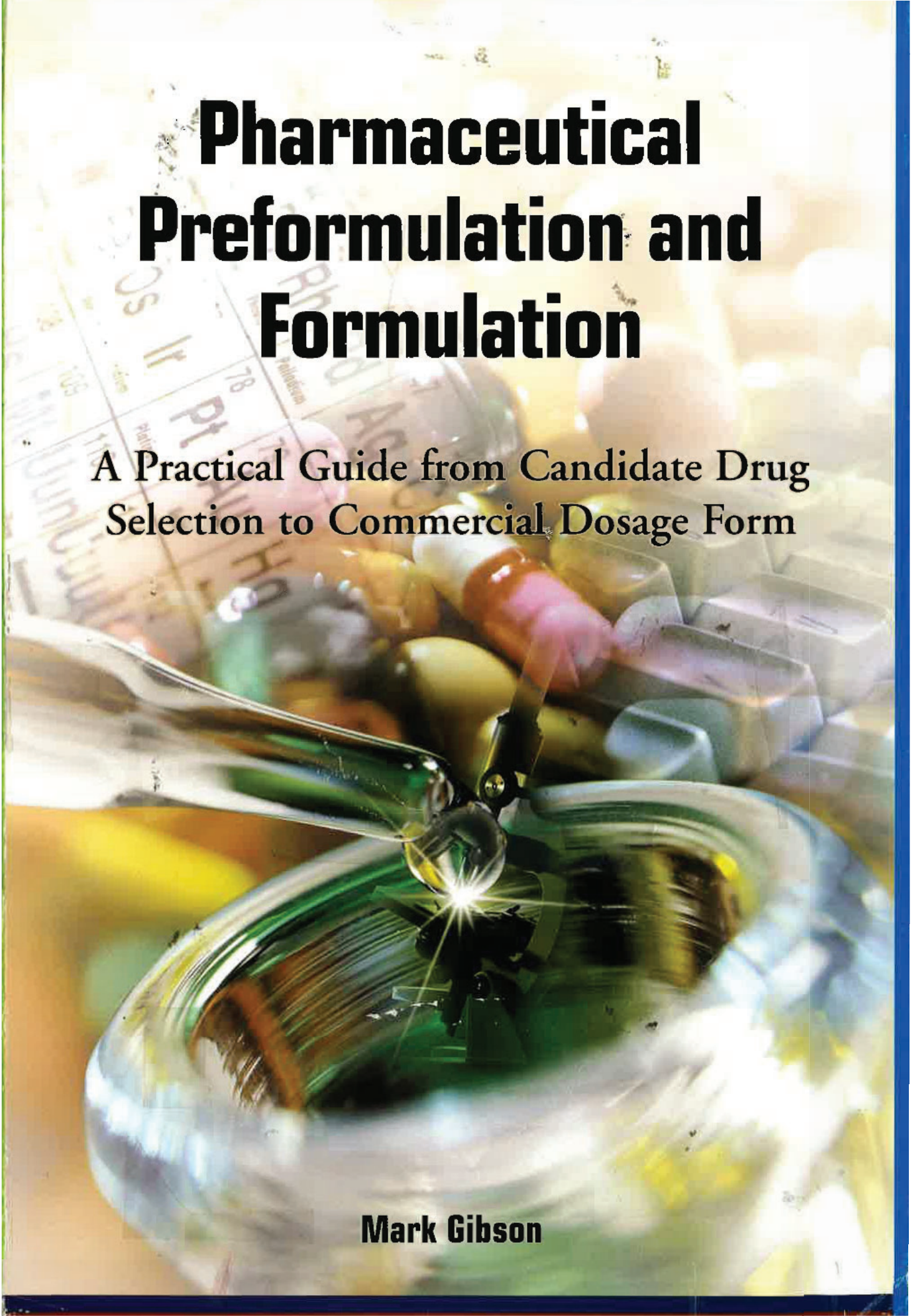
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Attachment A-9

Pharmaceutical Preformulation and Formulation

A Practical Guide from Candidate Drug Selection to Commercial Dosage Form



Mark Gibson

PHARMACEUTICAL PREFORMULATION AND FORMULATION

A Practical Guide from Candidate Drug Selection to Commercial Dosage Form

Mark Gibson
Editor

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IHS® Health Group
15 Inverness Way East
Englewood, CO 80112-5776, USA

Phone: +1-303-662-9101
Fax: +1-303-754-3953
Orders/on-line catalog:
www.ihshealthgroup.com

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1

Introduction and Perspective

Mark Gibson

AstraZeneca R&D Charnwood
Loughborough, United Kingdom

This book is intended to be a practical guide to pharmaceutical preformulation and formulation. It can be used as a reference source and a guidance tool for those working in the pharmaceutical industry or related industries, for example, medical devices and biopharmaceuticals, or anyone wanting an insight into this subject area. The information presented is essentially based on the extensive experiences of the editor and various other contributors who are all actively working in the industry and have learned "best practice" from their experiences.

There are various excellent books already available which cover the theoretical aspects of different types of pharmaceutical dosage forms and processes. A variety of books are also available that focus on the drug development process, business, regulatory and project management aspects. In my opinion, there has been a long-standing need for a pragmatic guide to pharmaceutical preformulation and formulation with an emphasis on what practical studies need to be undertaken, for what reasons and during what key stages of the drug development process. The important stages where preformulation, biopharmaceutics and formulation play a key role are candidate drug selection through the various stages of product development. This book has been written to try and address this need.

A logical approach to product development is described in the book, with the key stages identified and the preformulation, biopharmaceutics and formulation activities and typical issues at each stage discussed. Wherever possible, the book is illustrated with real or worked

examples from contributors who have considerable relevant experience of preformulation, biopharmaceutics and formulation development.

Jim Wells' book on preformulation (Wells 1988) made a strong impact on trainees and pharmaceutical scientists (including myself) working in this field of the pharmaceutical industry when it was introduced over 10 years ago. It describes the important concepts and methods used in preformulation with the underlying theory. To his credit, Wells' book is still useful today, but sadly, the book is now out of print, and existing copies are hard to obtain. It also requires updating to include modern preformulation instrumental techniques which have emerged over the last decade, such as thermo gravimetric analysis (TGA), hot stage microscopy (HSM), X-ray powder diffraction (XRPD), raman and infra-red spectroscopy and solid-state nuclear magnetic resonance (NMR), to name a few. These techniques can be used to provide valuable information to characterise the drug substance and aid formulation development using the minimal amounts of compound.

Pharmaceutical Preformulation and Formulation: A Practical Guide from Candidate Drug Selection to Commercial Formulation covers a wider subject area than just preformulation. Topics include biopharmaceutics, drug delivery, formulation and process development aspects of product development. The book also describes a logical and structured approach to the product development process, recommending at what stages appropriate preformulation, biopharmaceutics and formulation work is best undertaken.

DRUG DEVELOPMENT DRIVERS, CHALLENGES, RISKS AND REWARDS

It is important that the reader is aware of the nature of pharmaceutical research and development (R&D) in order to appreciate the importance of preformulation and formulation in the overall process.

In simple terms, the objective of *pharmaceutical R&D* can be defined as "converting ideas into candidate drugs for development", and the objective of *product development* defined as "converting candidate drugs into products for registration and sale". In reality, these goals are extremely challenging and difficult to achieve because of the many significant hurdles a pharmaceutical company has to overcome during the course of drug development. Some of the major hurdles are listed in Table 1.1.

The high risk of failure in drug discovery and development throughout the pharmaceutical industry statistically shows that, on average, only 1 in 5,000 to 1 in 10,000 compounds screened in research will reach the market (Tucker 1984). Of those that are nominated for development, the failure rate will vary from 1 in 5 to 1 in 10 compounds that will achieve registration and reach the market-place. On top of that, there is a significant commercial risk from those that are marketed; only 3 out of 10 are likely to achieve a fair return on investment. The products which give poor return on investment are often the result of poor candidate drug selection (the compound does not have the desired properties of safety, selectivity, efficacy, potency or duration) and/or poor product development (the development programme does not establish the value of the product). The latter scenario should, and can be, avoided by careful assessment at the "product design" stage of development. Product design is discussed further in Chapter 5.

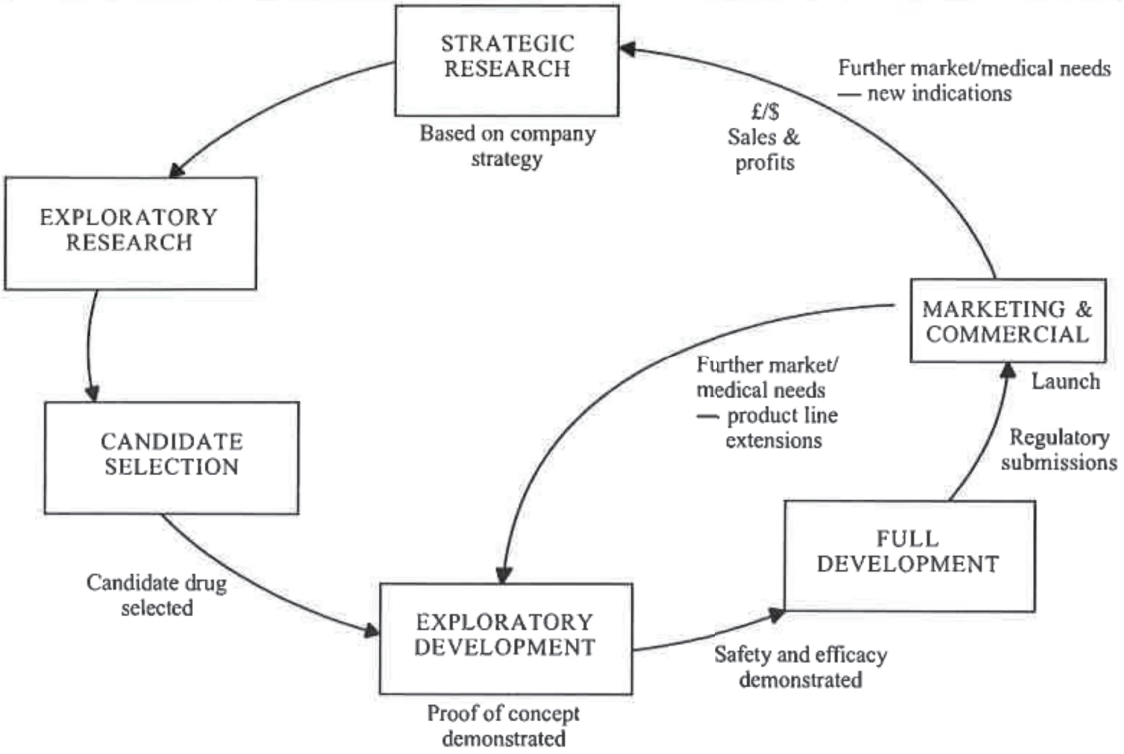
Table 1.1
Major hurdles to successful product registration and sale.

Activity	Requirements
Research	Novel compound (patentable?)
	Novel biological mechanism (patentable?)
	Unmet medical needs
	Potent and selective
Safety	High margin of safety
	Non-toxic (not carcinogenic, tetratogenic, mutagenic, etc.)
Clinical	Tolerable side-effects profile
	Efficacious
	Acceptable duration of action
Drug process	Bulk drug can be synthesised/scaled up
Pharmaceutical	Acceptable formulation/pack (meets customer needs)
	Drug delivery/product performance acceptable
	Stable/acceptable shelf-life
	Clinical trial process robust and can be scaled up
Regulatory	Quality of data/documentation
Manufacturing	Manufacturable
	Able to pass pre-approval inspection
Marketing/commercial	Competitive
	Meets customer needs
	Value for money
	Commercial return

To be successful and competitive, research-based pharmaceutical companies must ensure that new discoveries are frequently brought to the market to generate cash flow. This is required to fund the next generation of compounds to meet the therapeutic needs of patients, and of course, to benefit the shareholders. This cycle of events is sometimes referred to as the "product life cycle" and is further illustrated in Figure 1.1.

The costs of drug discovery and development to bring a New Chemical Entity (NCE) to the market are ever increasing. It is currently estimated that in excess of U.S. $500 million is required to recoup the costs of research, development, manufacturing, distribution, marketing and sales. A significant proportion of this total is for the cost of failures, or in other words, the elimination of unsuccessful compounds. R&D expenditure for an NCE tends to increase substantially as the compound progresses from drug discovery research through the various clinical trial phases of development. The pivotal Phase III patient trials are usually the largest, involving thousands of patients, and hence the most expensive. To reduce development costs,

Figure 1.1 Product life cycle.



some companies selectively screen and eliminate compounds earlier in the drug development process based on results from small-scale, less expensive studies in man and progress fewer, more certain compounds to later clinical phases.

In spite of the high risks and high costs involved, there is still a huge incentive for pharmaceutical companies to seek the financial rewards from successful marketed products, and especially from the phenomenal success of the rare "blockbuster" (reaching sales of >1 billion U.S.$ per year). This can earn the company significant profits to reinvest in research and fund the product development pipeline.

Another factor, the risk of delay to registration and launch, can also have a significant impact on the financial success of a marketed product. McKinsey & Company, a management consultancy, assessed that a product that is 6 months late to market will miss out on one-third of the potential profit over the product's lifetime. In comparison, they found that a development cost overspend of 50 percent would reduce profits by just 3.5 percent, and a 9 percent overspend in production costs reduced profits by 22 percent (McKinsey & Co. 1991). The loss of product revenue is often due to competitor companies being first to market, capturing the market share and dictating the market price, in addition to the loss of effective patent life. Hence, the importance of accelerating and optimising drug discovery and development, and getting to the market first with a new therapeutic class of medicinal product, cannot be underestimated. The second product to market in the same class will usually be compared with the market leader, often unfavourably.

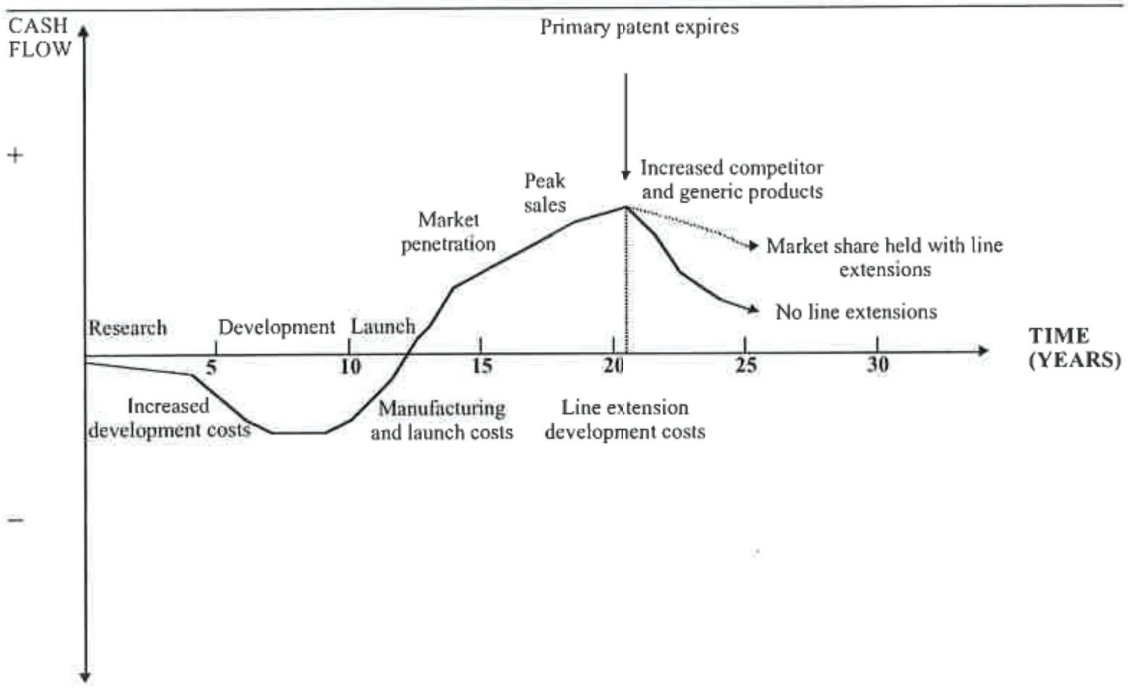
The average time from drug discovery to product launch is currently estimated to take 10 to 12 years. Several factors may have contributed to lengthening development times over the years, including an increase in the preclinical phase to select the candidate drug, and also an increase in the duration of the clinical and regulatory period required for marketing approval. Benchmarking studies show wide gaps between industry average or worst performance compared to what is achievable as best practice performance (Spence 1997). On average, the preclinical phase currently takes 4 to 6 years to complete, whereas the time from candidate drug nomination to regulatory submission takes on average 6 to 8 years, longer for treatments of chronic conditions. Most forward-looking pharmaceutical companies are aiming to reduce these times by re-evaluation and subsequently streamlining the development process, for example, by introducing more effective clinical programmes and more efficient data reporting systems, forward planning and conducting multiple activities in parallel. However, this, in turn, may put formulation development and clinical supplies on the critical path, with pressures to complete these activities in condensed time scales. Suggestions are offered throughout this book on how preformulation, biopharmaceutics and formulation can be conducted in the most efficient way to avoid delays in development times.

Any reduction in the total time-frame of drug discovery to market should improve the company's profitability. In a highly competitive market, product lifetimes are being eroded due to the pace of introduction of competitor products, the rapid introduction of generic products when patents expire and moves to "over-the-counter" (OTC) status. Successful pharmaceutical companies are focusing on strategies for optimum "product life cycle management" to maximise the early growth of the product on the market, sustain peak sales for as long as the product is in patent and delay the post-patent expiry decline for as long as possible. This should maximise the return on investment during a product life cycle to enable the company to recover development costs and make further investments in R&D. Figure 1.2 shows a classic cash flow profile for a new drug product developed and marketed. During development there is a negative cash flow, and it may be some time after launch before sales revenue crosses from loss to profit because of manufacturing, distribution and advertising costs. Profits continue to increase as the market is established to reach peak sales, after which sales decrease, especially after the primary patent expires and generic competition is introduced.

Throughout the life span of a product, it is in a company's interest to ensure the best patent protection in order to achieve the longest possible market exclusivity. Prior to the primary patent expiring (normally for the chemical drug substance), it is imperative to introduce new indications, formulations, manufacturing processes, devices and general technology, which are patent protected, to extend the life of the product and maintain revenue. A patent generally has a term of about 20 years, but as development times are getting longer, there will be a limited duration of protection remaining once the product is marketed (the effective patent life). A comparison of effective patent life for pharmaceutical new chemical entities in various countries around the world shows the same downward trend between the 1960s and the 1980s (Karia et al. 1992; Lis and Walker 1988).

Getting to the market quickly is a major business driving force, but this has to be balanced with the development of a product of the appropriate quality. There is a need to generate sufficient information to enable sound decisions on the selection of a candidate drug for development, as well as to develop dosage forms which are "fit for purpose" at the various stages of development. Anything more is wasting precious resources (people and drug substance), adding unnecessary cost to the programme and, more importantly, extending the development time. Perfect quality should not be the target if good quality is sufficient for the intended purpose. This can only be achieved if there is a clear understanding of the customer requirements.

Figure 1.2 Product life cycle management.



For example, if a simple, non-optimised formulation with a relatively short shelf life is acceptable for Phase I clinical studies, any further optimisation or stability testing might be considered wasteful, unless the data generated can be used later in the development programme.

There can be a significant risk associated with doing a minimum development programme and cutting corners to fast track to market. Post-launch, the cost of a retrospective fix due to poor product/process design and/or development can be extremely high. The additional financial cost from work in product/process redevelopment, manufacturing and validation, technical support, regulatory and sales and marketing (due to a product recall) can easily wipe out the profit from an early launch. This can have several unpleasant knock-on effects; it may affect the market share and the company's relationship with the regulatory authorities, and its credibility with customers (both externally and internally within the company) may be threatened. These factors need to be taken in to account when planning preformulation/formulation studies which can directly influence the progress of a product to market and final product quality.

CURRENT TRENDS IN THE PHARMACEUTICAL INDUSTRY

Increasing competition and threats to the pharmaceutical industry with respect to maintaining continued sales growth and income mean that successful companies going forward will be those which have a portfolio of products capable of showing volume growth. However, to show volume growth innovative new products are required. The cost of drug discovery and development is escalating because there are no easy targets left and the cost of development

and the cost of goods sold is increasing. There have been several mergers and acquisitions of research-based pharmaceutical companies, and increased collaborations and inward licensing of products and technologies, in attempts to acquire new leads, to share costs, to reduce the time to licence and to maintain growth. Unfortunately, mergers and acquisitions also result in streamlining and job losses which improve efficiency and decrease overhead costs at the same time.

There is a changing trend in the nature of the candidate drug emerging from pharmaceutical R&D, from a low molecular weight chemical to a more complex macromolecule (biologicals), which can be a peptide, protein, enzyme, antibody, nucleic acid, genetic material or a multicomponent vaccine. Some of these compounds have been derived from biotechnological processes to produce biotechnological medicinal products that fight infection and disease. The U.S. Food and Drug Administration (FDA) and European Agency for the Evaluation of Medicinal Products (EMEA) have already approved biotechnological medicinal products for anaemia, cystic fibrosis, growth deficiency, hepatitis and transplant rejection. Many more are being developed to treat cancer, human immunodeficiency virus (HIV) infections and acquired immunodeficiency syndrome (AIDS), multiple sclerosis and stroke. A major challenge to the formulator is to develop self-administered formulations to deliver macromolecules such as proteins and polypeptides into the body, for example, by the oral or inhalation route.

More sophisticated drug delivery systems are being developed to overcome the limitations of conventional forms of drug delivery systems (e.g., tablets and intravenous [IV] solutions), to overcome problems of poor drug absorption, the non-compliance of patients and inaccurate targeting of therapeutic agents. One example of an emerging drug delivery technology is the use of low-level electrical energy to assist the transport of drugs across the skin in a process known as electrophoresis. This method could be particularly useful for the delivery of peptides and proteins which are not adequately transported by passive transdermal therapy. The drug absorption rate is very rapid and more controlled compared with passive diffusion across the skin. Another example is the improvement in inhaler technology to ensure a more efficient delivery to the lungs, with minimal drug deposition in the mouth and trachea. The use of a breath-actuated aerosol is designed to co-ordinate drug delivery with the patient's inhalation to achieve this. A third example is the use of bioerodable polymers that can be implanted or injected within the body to administer drugs from a matrix which can be formulated to degrade over a long duration from one day to six months, and do not require retrieval. Some of these specific delivery systems are explained in more detail in later chapters of this book on the various dosage forms.

Futuristic drug delivery systems are being developed which hope to facilitate the transport of a drug with a carrier to its intended destination in the body and then release it there. Liposomes, monoclonal antibodies and modified viruses are being considered to deliver "repair genes" by IV injection to target the respiratory epithelium in the treatment of cystic fibrosis. These novel drug delivery systems not only offer clear medical benefits to the patient but can also be opportunities for commercial exploitation, especially useful if a drug is approaching the end of its patent life.

There are pressures on the pharmaceutical industry which affect the way products are being developed. For example, there is a trend for more comprehensive documentation to demonstrate compliance with current Good Manufacturing Practice (cGMP) and Good Laboratory Practice (GLP) and to demonstrate that systems and procedures have been validated. The trend is for more information required for a regulatory submission, with little flexibility for changes once submitted. Therefore, the pressure is for a company to submit early and develop the product "right first time".

In spite of efforts to harmonise tests, standards and pharmacopoeias, there is still diversity between the major global markets—Europe, the United States and Japan—which have to be taken in to account in the design of preformulation and formulation programmes (Anonymous 1993). This is discussed further in Chapter 5 on product design.

Other pressures facing the pharmaceutical industry are of a political/economical or environmental nature. Some governments are trying to contain healthcare costs by introducing healthcare reforms, which may lead to reduced prices and profit margins for companies, or restricted markets where only certain drugs can be prescribed. Although the beneficial effect of drugs is not questioned in general, the pressure to contain the healthcare costs is acute. Healthcare costs are increasing partly because people are living longer and more treatments are available. This may influence the commercial price that can be obtained for a new product entering the market and, in turn, the "cost of goods (CoG) target". The industry average for the CoG target is 5 to 10 percent of the commercial price with pressure to keep it as low as possible. This may impact on the choice and cost of raw materials, components and packaging for the product and the design and cost of manufacturing the drug and product.

Environmental pressures are to use environmentally friendly materials in products and processes and to accomplish the reduction of waste emissions from manufacturing processes. A good example is the replacement of chlorofluorocarbons (CFCs) propellants in pressurised metered dose inhalers (pMDIs) with hydrofluorocarbons (HFAs). The production of CFCs in developed countries was banned by the Montreal Protocol (an international treaty) apart from "essential uses", such as propellants in pMDIs, to reduce the damage to the earth's ozone layer. However, there is increasing pressure to phase out CFCs altogether. The transition from CFC to HFA products involves a massive reformulation exercise with significant technical challenges and costs for pharmaceutical companies involved in developing pMDIs, as described in Chapter 10 "Inhalation Dosage Forms". However, this can be turned into a commercial opportunity for some companies which have developed patent-protected delivery systems to extend the product life cycle of their CFC pMDI products.

LESSONS LEARNT AND THE WAY FORWARD

To achieve the best chance of a fast and efficient development programme to bring a candidate drug to market, several important messages can be gleaned from projects which have gone well and from companies with consistently good track records.

There are benefits for pharmaceutical development to get involved early with preclinical research during the candidate drug selection phase. This is to move away from an "over-the-wall" hand-over approach of the candidate drug to be developed from "research" to "development". The drug selection criteria will be primarily based on pharmacological properties such as potency, selectivity, duration of action and safety/toxicology assessments. However, if all these factors are satisfactory and similar, there may be an important difference between the pharmaceutical properties of candidate drugs. A candidate drug with preferred pharmaceutical properties, for example, good aqueous solubility, crystalline, nonhygroscopic and good stability, should be selected to minimise the challenges involved in developing a suitable formulation. This is discussed further in Chapter 2.

Another important factor is good long-term planning, ideally from candidate drug nomination to launch, with consideration for the safety, clinical, pharmaceutical development, manufacturing operations and regulatory strategies involved to develop the product. There is a need for one central, integrated, company project plan that has been agreed on by all parties

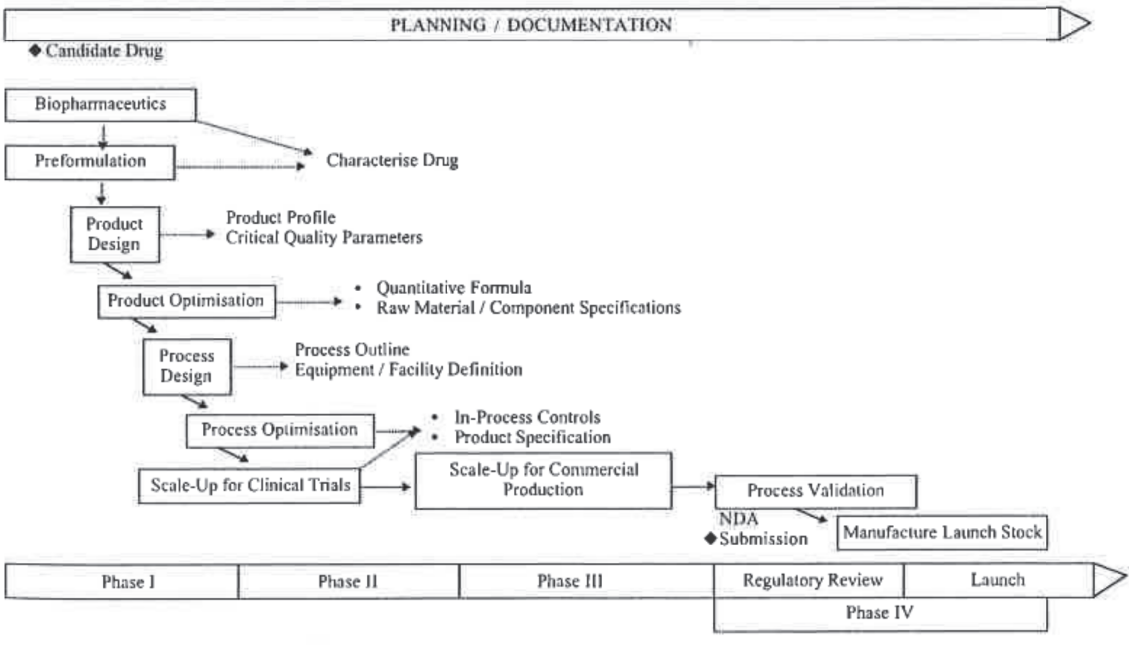
with a vested interest in the project. Needless to say, the plan should contain details of activities, timings, responsibilities, milestones, reviews and decision points. Reviews and decision points are required at the end of a distinct activity to ensure that the project is still meeting its objectives and should progress to the next stage of development. However, these reviews should not cause any delays to the programme, rather, they should ratify what is already progressing. The traditional sequential phases of product development (see Chapter 2) must be overlapped to accelerate the product to market. In reality, plans will inevitably change with time; they should be "living" documents which are reviewed and updated at regular intervals and then communicated to all parties. There may be several more detailed, lower-level plans focusing on departmental activities, e.g., for pharmaceutical development, but these plans must be linked to the top level central project plan.

Forward planning should provide the opportunity for a well thought out and efficient approach to product development, identifying requirements up front so as to avoid too much deliberation and backtracking along the way. It also should provide a visible communication tool.

Good planning is supported by adopting a systematic and structured approach to product development. The development process can be broken down into several key defined stages—product design, process design, product optimisation, process optimisation, scale-up and so on. Each stage will have inputs and outputs as shown in Figure 1.3, a simplified framework for product development. The appropriate definition and requirements at each stage are described in Chapters 5 and 8 of this text.

As product development can take several years to complete, it is important to have an effective document management system in place to record the work. The primary reference source for recording experimental work will usually be a laboratory notebook. The work should be checked, dated and counter-signed to satisfy GLP and intellectual property requirements. Experimental protocols are sometimes useful for defining programmes of work,

Figure 1.3 Framework for product development.



explaining the rationale for the studies and defining the acceptance criteria. When the studies are completed, the results can be reported with reference to the protocol and acceptance criteria. Laboratory notebooks are referenced in the protocols and reports so that the raw data can be retrieved in the event of an audit.

At the completion of key stages of the work, summary reports can be written, referencing all other protocols and reports relevant to that stage and highlighting the major recommendations and conclusions. In this way, a product development document file can be built up for transfer of information and technology, including the development history and rationale for progression. The file will also be vital for data retrieval in the event of a regulatory inspection.

Finally, successful product development is often associated with good teamwork. The process is multidisciplinary, relying on people with different specialist skills working together to make it happen. This is particularly important at the key interfaces such as preclinical research with pharmaceutical development and pharmaceutical development with manufacturing operations at the final production site. It is therefore useful to have representation on the project teams from all the key specialist functions to ensure buy-in to the plans, strategies and decisions and to have a good project management system in place.

SCOPE OF THE BOOK

This book is structured in a logical order to cover the various stages of drug development from candidate drug selection to development of the intended commercial dosage form.

In Chapter 2, the key stages of the R&D process are explained in some detail, with the outputs expected from each stage, to afford an appreciation of the entire process. The remainder of the book concentrates on candidate drug selection for development and development of the commercial dosage form where preformulation, biopharmaceutics and formulation play a vital role. Initial emphasis is on candidate drug selection and the importance of preformulation, formulation and biopharmaceutics input at this stage. Traditionally, not all pharmaceutical companies operate in this way, and the result from experience is often that pharmaceutical development has to accept whatever candidate drug comes out of research and address any unforeseen difficulties during development. The disadvantages of this approach, and the opportunities and benefits of pharmaceutical input to the candidate selection process, are clearly explained in the early chapters.

Available drug substance for preformulation and biopharmaceutics studies at the candidate drug selection stage can be a major challenge. Chapter 3 describes the preformulation studies that can be undertaken to maximise the information gained from small amounts of drug substance in order to select the preferred candidate drug for development. Various modern preformulation techniques which use minimal amounts of drug are described to evaluate the physicochemical properties of compounds, salts and polymorphs.

Chapter 4 describes the importance of drug delivery and biopharmaceutical factors in the candidate drug selection phase. Consideration is given to the intended route of administration and what predictions can be made and useful information gained from biopharmaceutical assessment of the candidate drug.

Following candidate selection, usually one candidate drug is nominated for development. The importance of establishing the product design attributes are discussed in Chapter 5. The value of this exercise is often underestimated in the rush to develop products quickly. However, the quality of the product design can often influence the success of developing a commercially viable product with a desired product profile in a timely manner to market.

Chapters 6 and 7 focus on preformulation and biopharmaceutics, respectively, as an aid to product design. The emphasis is on generating the appropriate data to characterise the candidate drug and aid product design and development. The objective at this stage is to determine the physicochemical properties of the candidate drug which are considered important in the development of a stable, effective and safe formulation. Limited availability of drug substance, speed and careful consideration of the programme of work depending on the intended dosage form and route are all considered here and illustrated with the aid of worked examples. Modern instrumental techniques and personal computer (PC)–based "expert systems" are discussed as useful tools.

To develop a product from inception to market, the product and process have to be optimised and the process scaled up and transferred to commercial production. Definitions and descriptions of the requirements for all these stages of development are discussed in Chapter 8, although the major discussion is on the preformulation/formulation input to product optimisation. The many factors which a formulator should consider in the selection of pharmaceutical excipients and packaging are discussed. Useful sources of information and techniques for selection such as expert systems and experimental design tools are included.

Drugs are generally administered via the mouth, eyes, nose, skin, or by inhalation or injection, and so these routes are covered in more detail in separate chapters of this book. Special considerations and issues for the formulation development of each route and type of dosage form are discussed, based on the considerable relevant experience of the various contributors.

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About This Book

Successful pharmaceutical companies must balance [illegible]rket with development of products of high quality. Striking the corre[illegible]unt when planning preformulation/formulation studies, studies that dir[illegible]ducts to market. In addition, consideration must be given to regulatory requirements such as compliance with GLP and GMP, validation of systems and procedures, and comprehensive documentation. In *Pharmaceutical Preformulation and Formulation: A Practical Guide from Candidate Drug Selection to Commercial Dosage Form,* Mark Gibson has answered the need for a practical reference source and pragmatic guide on what studies need to be undertaken, for what reasons, and at what key stages of the drug development process. In addition to preformulation, his book covers biopharmaceutics, drug delivery, formulation, and process development aspects of product development.

Ten contributing authors have shared their experience and expertise in significant chapters divided into three useful sections: *Aiding Candidate Drug Selection, Early Drug Development,* and *From Product Design to Commercial Dosage Form.* This book should be especially useful to scientists, technicians, and managers in the pharmaceutical, medical device, and biopharmaceutical industries.

What You'll Learn

- early preformulation/formulation and biopharmaceutical information to aid in selection of the most suitable compound for development
- prediction of potential problems for further formulation development
- best use of drug substance for preformulation studies to aid candidate drug selection
- generation of appropriate preformulation/formulation data to characterize the candidate drug and aid product design and development
- instrumental techniques that use minimal amounts of drug substance to gain useful information
- PC based Expert systems to aid product design
- various dosage forms
- routes of drug administration
- pros and cons of contracting out development

About the Editor

Mark Gibson, BPharm, PhD, CChem, MRSC, MRPharmS, is responsible for parenteral and oral solid dosage form development at AstraZeneca R&D Charnwood, a division of AstraZeneca, in the UK. His experience includes formulation and preformulation development, both as a bench scientist and a manager, at Cyanamid (Lederle), Fisons Pharmaceuticals, Astra Pharmaceuticals, and AstraZeneca. He has worked on a variety of dosage forms and routes of delivery, including inhalation, oral, nasal, ophthalmic, parenteral, and transdermal, some of which have resulted in patents and marketable products. Dr. Gibson is a member of the UK Academy of Pharmaceutical Sciences, the Parenteral Society, and the Aerosol Society.

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CERTIFICATE OF SERVICE

I hereby certify that, on August 17, 2023, I caused a true and correct copy of the following materials:

- Patent Owner's Supplemental Evidence in Response to Petitioner's Objections to Evidence Under 37 C.F.R. § 42.64

to be served via email on the attorneys identified in the Petition for Petitioner Apotex, Inc. (Paper No. 1), whom consented to electronic service:

Vishal C. Gupta (Reg. No. 67,284)
John J. Molenda (Reg. No. 47,804)
Tyler Doh (Reg. No. 80,274)
Steptoe & Johnson LLP
1114 Avenue of the Americas
New York, NY 10036
Tel: (212) 506-3900
Fax: (212)-506-3950

Robert Kappers (Reg. No. 70,202)
Steptoe & Johnson LLP
227 West Monroe Street
Suite 4700
Chicago, IL 60606
Tel: (312)-577-1300

Azacitidine@Steptoe.com

/Gabriel J. Rosanio/
Gabriel J. Rosanio (Reg. No. 81,426)
Wilmer Cutler Pickering Hale and Dorr LLP
7 World Trade Center
250 Greenwich Street
New York, NY 10007
Tel: 212-295-636