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Exhibit 1029

**THE UNITED STATES PATENT AND TRADEMARK OFFICE**

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| Applicant: | Sharon Erickson | Attorney Docket #: | GNE-0073R2C2 |
| Serial No. | 11/949,351 | Group Art Unit | 1643 |
| Filing Date | 12/03/2007 | Examiner: | Natarajan, Meera |
| Customer No.: | 35489 | Confirmation No.: | 4598 |
| Title: | **METHODS OF TREATMENT USING ANTI-ErbB ANTIBODY MAYTANSINOID CONJUGATES** | | |

**FILED VIA EFS**

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

# DECLARATION OF BARBARA KLENCKE, M.D.

I, Barbara Klencke, M.D., declare and say as follows: -

1. I obtained a B.S. in 1979 from Indiana University, and an M.D. from University of California, Davis. I completed my residency at University of California San Francisco (UCSF) Medical Center. Following two years of Medical Oncology fellowship training at UCSF, I served as Assistant Professor of Medicine for over seven years at UCSF.

2. I joined Chiron (later acquired by Novartis) in 2002 and served as Medical Director for one year, and in 2003, I joined Genentech, Inc., where I worked as Associate Group Director, and where my current title is Group Director.

3. During my employment at Genentech, I have worked on the development of marketed products, such as Avastin® and Tarceva®, and several therapeutic compounds in early development, prior to working on the development of T-DM1. T-DM1 is a therapeutic immunoconjugate in which the anti-ErbB2 antibody trastuzumab is conjugated to the cytotoxic maytansinoid "DM1." Part of my responsibilities as Associate Group Director included the

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development of clinical trial strategy for T-DM1 in the treatment of tumors, primarily breast tumors, that express ErbB2.

4. My Curriculum Vitae, including my list of publications, patents, pending patent applications, and awards, is enclosed as **Exhibit A** and forms part of this Declaration.

5. I am familiar with and understand the disclosure of the above-identified patent application, including the currently pending claims, including the new claims added concurrently with filing the present Declaration. I am also familiar with and understand the Office Action mailed on June 8, 2010 in connection with the above-identified patent application, and the references cited in that Office Action. Claim 40 is directed to an immunoconjugate comprising the antibody huMAb4D5-8 conjugated to a maytansinoid. The antibody huMAb4D5-8 is the same antibody as trastuzumab and is marketed under the tradename Herceptin®. All other claims depend, directly or indirectly, from claim 40.

6. According to the Office Action, the claimed invention would have been obvious to one of ordinary skill in the art at the time the invention was made over the combination of U.S. Patent No. 5,208,020 (referred to hereinafter as "Chari et al.") and U.S. Patent No. 6,054,297 (referred to hereinafter as "Carter et al."). Chari et al. allegedly teach a composition comprising one or more maytansinoids (col. 6-8) linked to a monoclonal antibody or antibody fragment, where the monoclonal antibody is selective for tumor cell antigens. Carter is cited for disclosing humanized 4D5 antibodies, including huMAb4D5-8, and for allegedly teaching that the humanized 4D5 antibodies may be used as immunotoxins, conjugated with a cytotoxic moiety (col. 44). The Examiner acknowledges that Chari et al. do not teach an anti-ErbB2 antibody conjugated to a maytansinoid. The finding of obviousness is based on the assertion that one of ordinary skill would have been motivated to make the claimed antibody-maytansinoid conjugates because such conjugates fall within the scope of the immunotoxins of Carter et al. and because Carter et al. teach that ErbB2 is amplified or overexpressed in human malignancies.

7. Before the earliest priority date of the present application (March 16, 2000), it was known that ErbB2 (also known as HER2) is amplified and overexpressed in certain breast and ovarian cancers, and such overexpression is correlated with poor prognosis. (See application at page 2, para. [0006].) It was also known that HER2 is expressed in normal tissues at levels



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similar to those found in non-HER2-amplified, non-HER2-overexpressing breast cancers and breast cancer cell lines. For example, HER-2 protein was identified on cell membranes of normal epithelial cells in the gastro-intestinal, respiratory, reproductive, and urinary tract as well as in the skin, breast and placenta, demonstrating that HER2 is normally a membrane constituent of a variety of epithelial cell types. (See, Press et al., *Oncogene* 5(7):953-62, 1990 - **Exhibit B,** Abstract, Table 1 and Figures 1 and 2.)

8. Herceptin® was approved by the FDA in 1998 for the treatment of metastatic HER2-overexpressing breast cancer, either as an initial treatment in combination with chemotherapy (paclitaxel), or as a monotherapy after prior treatment with chemotherapy. Herceptin® was later approved in 2006 as part of a multi-agent regimen for the adjuvant (post-surgical) treatment of HER2-overexpressing breast cancer. In 2008, Herceptin® received further approvals from the FDA for treatment in the adjuvant setting. Although Herceptin® is a breakthrough in the treatment of HER2-overexpressing ("HER2-positive") breast cancer, nearly all patients with metastatic disease treated with Herceptin® will progress after experiencing a period of clinical benefit, and there remains an unmet medical need for patients with HER2-positive breast cancer. (See application at page 5, para. [0014].)

9. The maytansinoids are a family of cytotoxic molecules that include maytansine and its derivative DM1. Before the priority date, it was reported that maytansine acts as a very potent mitotic inhibitor by inhibiting microtubule polymerization. (See abstract, Rao et al., *Cancer Research* 39:3152-3155, 1979 – **Exhibit C**; and see abstract, Remillard et al., *Science* 189:1002-1005, 1975 – **Exhibit D**.) Maytansine was also reported as being of relatively high cytotoxicity (i.e., about 100-200 fold more cytotoxic than other anti-mitotic agents). (See **Exhibit C**, col. 1, para. 2.) Maytansine was unsuccessful in human clinical trials because of unacceptable systemic toxicity, i.e., maytansine did not demonstrate clinically reasonable therapeutic benefit at safe doses. (See Issell et al., *Cancer Treat. Rev.* 5:199-207, 1978 – **Exhibit E**, e.g., at pages 204 and 207.)

10. Before the earliest priority date of the present application, it was recognized in the art that some monoclonal antibody-based therapies had begun to show promise in the treatment of cancer, as demonstrated, e.g., by Herceptin® and by Rituxan®, another Genentech monoclonal



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antibody first approved in 1997 for the treatment of non-Hodgkin's lymphoma. Despite the promise shown by some monoclonal antibodies in the clinical setting at the priority date, immunoconjugates (referring to antibodies conjugated to moieties such as radioisotopes, enzymes, drugs, and cytotoxic agents) had achieved very limited clinical success at that time, a fact that holds true to this day. Indeed, Trail and Bianchi recognized at the priority date that immunoconjugates were not "established" chemotherapeutic agents and that further work in the field was needed to improve "the efficacy and therapeutic index of immunoconjugates by optimizing selectivity and potency." (See Trail and Bianchi, *Current Opinion in Immunology* 11:584-588, Oct. 1999 - **Exhibit F**, e.g., at page 586, col. 2, para. 2.) "Selectivity" refers to the ability of immunoconjugates to bind preferably to tumor cells expressing the target antigen over normal cells that may also express the target antigen, while "potency" refers to the degree of cytotoxicity of the immunoconjugate, as conferred by the cytotoxic moiety of the immunoconjugate. Trail and Bianchi cautioned that "[i]t is therefore necessary to balance the relative selectivity of the MAb [monoclonal antibody] with the potency of the agent delivered." (See **Exhibit F** page 584, col. 1, para. 2.) If that balance is not achieved, an immunoconjugate may cause toxicity on the one hand, or may not be sufficiently potent on the other. (See **Exhibit F**, page 585, col. 1, first para., and col. 2, last para.) Indeed, Trail and Bianchi further cautioned that "*even low levels of expression of the targeted antigen by normal cells may lead to significant toxicity.*" (**Exhibit F**, page 585, col. 1, para. 1, emphasis added.)

11. Thus, at the priority date, the immunoconjugate field was still evolving with no outstanding successes, and there was considerable uncertainty and concern surrounding the therapeutic potential and in particular, the therapeutic index, of immunoconjugates. Indeed, Trail and Bianchi predicted that the major role of immunoconjugates would lie in "minimal disease settings," based on the assumption that potency would need to be comprised in order to avoid unacceptable levels of toxicity. (See **Exhibit F**, e.g., at page 585, col. 2, last para, and page 586, col. 2, last para.)

12. In my capacity as Associate Group Director at Genentech, I played a major role in designing the "TDM4374g" clinical trial protocol. The TDM4374g clinical trial is a Genentech/Roche-sponsored single-arm, multi-center Phase II trial of T-DM1 in 110 patients with HER2-positive metastatic breast cancer. Patients that participated in this study had



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Exhibit 1029-04

progressive disease after having been treated with at least the following five different agents or classes of agents: an anthracycline, a taxane, capecitabine (Xeloda®) and two HER2-directed therapies, i.e., Herceptin® and lapatinib (Tykerb®, an inhibitor of the intracellular tyrosine kinase domain of HER2). Anthracyclines, taxanes and capecitabine encompass the most commonly used chemotherapeutic agents in the treatment of breast cancer; Herceptin® and lapatinib represent the *only* FDA-approved HER2-directed therapies for metastatic HER2-positive breast cancer. Thus, the patients in the TDM4374g trial had been previously treated with at least five agents representing the most commonly used classes of drugs currently available for the treatment of metastatic breast cancer, including the only two that are approved for the treatment of HER2-positive breast cancer.

13. The patients enrolled in the TDM4374g trial had been treated with a median of seven different agents, yet their cancers had progressed after the last therapy received. T-DM1 was essentially a last option for the terminally ill patients in this study.

14. At this point in the TDM4374g trial, the clinical activity of T-DM1 has well exceeded what a clinical oncologist practicing in the field of metastatic breast cancer would have expected, given the clinical setting the trial was designed to address as well as the activity of other agents currently used in the treatment of metastatic breast cancer.

15. In the TDM4374g trial as of December 2009, the objective response rate (ORR), which is the percentage of patients whose tumors shrank by at least 30% after treatment with T-DM1, was 32.7%, as determined by an independent review facility. The clinical benefit rate, which is the percentage of patients with objective response or stable disease maintained for at least 6 months, was 44.5%, as determined by an independent review facility.

16. To put these data in perspective, patients initially presenting with HER2-positive metastatic breast cancer typically receive Herceptin® in combination with a taxane. If a patient's disease progresses after that "first-line" therapy, then a "second-line" HER2-directed therapy is typically administered. That second-line therapy typically consists of either Herceptin® or lapatinib administered in combination with further chemotherapeutic agents. After first- and second-line therapies fail, the treatment options for metastatic breast cancer are very limited.



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One remaining approved treatment option in this "third-line" setting is ixabepilone (Ixempra®; Bristol–Myers Squibb), a non-HER2-specific microtubule inhibitor.

17. The ORR for T-DM1 was unexpectedly better than the ORR seen with agents currently used in second- and third-line metastatic breast cancer therapies. For example, the ORR for the combination of lapatinib and capecitabine was 23.7% in a "second-line" study of patients that had received prior treatment with an anthracycline, a taxane and Herceptin®. (See Tykerb® package insert, rev. 2010 – **Exhibit G**, e.g., at page 15, Table 5.) The ORR for ixabepilone in the treatment of metastatic breast cancer previously treated with an anthracycline, a taxane and capecitabine was 12.4%. (See Ixempra® package insert, rev. 2009 – **Exhibit H**, e.g., at Table 8.)

18. Based on my experience as an oncologist, the trial would have been considered a success if T-DM1, which was administered as a third-line therapy in TDM4374g, demonstrated an ORR comparable to that of current second line therapies. Thus, an ORR of about 24% would have been considered a favorable outcome in the TDM4374g trial. The fact that the ORR of T-DM1 in TDM4374g (32.7%) significantly exceeded that of current second-line therapies (23.7%), and well surpassed that of current third line therapies (12.4%), was a better result than expected.

19. T-DM1 also showed considerably low systemic toxicity. T-DM1 was generally well tolerated by patients at the dose and schedule tested, and the observed toxicities were generally acceptable and manageable in this patient population. The low toxicity of T-DM1 is an advantage, particularly in light of the fact that HER2 is expressed on normal cells, and that maytansine (from which DM1 is derived) had demonstrated unacceptable toxicity in previous clinical trials.

20. It is my considered opinion that T-DM1 provides an important advance in the field of HER2-positive metastatic breast cancer treatment, by showing unexpectedly superior activity in a patient population that had progressed after treatment with multiple standard chemotherapies as well as all currently approved HER2-directed therapies. TDM4374g is the first clinical trial that specifically treated patients who had progressive disease after receiving five FDA-approved agents or classes of agents for the treatment of breast cancer, including the



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only two approved HER2-directed agents (trastuzumab and lapatinib). That patient population has very limited treatment options. Current chemotherapeutic options in this setting have poor response rates and significant toxicity. In contrast, T-DM1 provides a viable single treatment option with impressive efficacy and a favorable safety profile.

21. The opinion that T-DM1 demonstrated unexpectedly superior activity is reflected by the statements of others in the field praising the performance of T-DM1 in the TDM4374g trial. For example, Dr. Ian Krop, the lead clinical investigator in the study, was quoted in a "WebMD" online article as stating that "[t]his is the first study looking at women who have failed so many other treatments" and "we think *these results are as good as we've ever seen in such a refractory population.*" (See WebMD website http://www.webmd.com/breast-cancer/news/20091215/targeted-breast-cancer-drug-shrinks-tumors, dated 12/15/2009, printed on 12/18/2009 – **Exhibit I**, emphasis added.) Dr. Krop was also quoted in an online article in "TheStreet.com" as stating: "T-DM1 is a drug that I'd like to see made available to patients. I'm sure Genentech [Roche] is strongly considering *using this data for the basis of an accelerated approval filing.*" (See http://www.thestreet.com/print/story/10644637.html, dated 12/14/2009, printed on 12/18/2009 – **Exhibit J**, emphasis added.)

22. Edith Perez, M.D., a breast cancer specialist and internationally known researcher at the Mayo Clinic, was quoted in the "WebMD" online article cited above as stating that "[t]he *response rate they saw in the study is exceptional* in a group of patients this ill." (See **Exhibit I,** emphasis added.) (It is noted that Dr. Perez was not a clinical investigator in the TDM4374g trial but is an investigator in other Genentech/Roche-sponsored clinical trials of T-DM1 in other settings.)

23. It is also my considered opinion that the results of the TDM4374g trial challenged the skepticism surrounding immunoconjugates at the priority date, e.g., that the major role of immunoconjugates would lie "in minimal disease settings." In the TDM4374g trial, T-DM1 demonstrated unexpectedly superior efficacy as a single agent in an extremely advanced disease setting, thus well exceeding expectations at the priority date. T-DM1 achieved this feat by demonstrating both impressively high potency and low toxicity. Those results were not



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predictable, given that maytansinoids are highly cytotoxic molecules and that HER2 is expressed on normal cells.

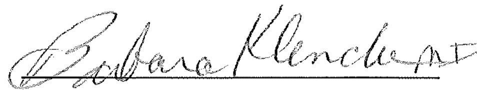
24. It is further my considered opinion that T-DM1 fills a long felt but unresolved need at the priority date, i.e., the need for HER2-directed agents that treat metastatic HER2-positive breast cancer. At the priority date, only one HER2-directed therapy, i.e., Herceptin®, had been approved by the FDA for the treatment of HER2-overexpressing metastatic breast cancer. Moreover, nearly all patients with metastatic disease treated with Herceptin® will progress after experiencing a period of clinical benefit.

25. The opinions expressed in paragraphs 20 and 23-24 are consistent with those of other experts in the field, who have praised T-DM1 and would like to see T-DM1 available to patients (i.e., approved by the FDA) as soon as possible. As shown by the results of the TDM4374g trial, T-DM1 has the potential to considerably improve the lives of breast cancer patients, offering new hope to patients suffering with advanced, aggressive disease.

26. It is also my considered opinion that T-DM1 is a pioneer in the immunoconjugate field. To this day (about 10 years after the priority date), immunoconjugates have achieved very limited clinical success. Although immunoconjugates have been subject to over two decades of research and development within the scientific and pharmaceutical community, the FDA has thus far approved only one immunoconjugate, Mylotarg®, for the treatment of acute myeloid leukemia, a hematologic cancer. The unexpectedly superior activity of T-DM1 in treating solid tumors represents an important advance in the field of immunoconjugates.

27. I declare further that all statements made in this Declaration 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 are made with the knowledge that willful 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 and that such willful false statements may jeopardize the validity of the application or any patent granted thereon.

Dated: June 28, 2010



BARBARA KLENCKE, M.D.



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# EXHIBIT A

**Barbara J. Klencke, M.D.**

Genentech, Inc.
1 DNA Way, M/S 66
South San Francisco, CA 94080
(650) 467-6278 Phone
e-mail: bklencke@gene.com

## *Employment*

2010 - present Group Medical Director
2003 - 2010 Associate Group Medical Director
BioOncology
Genentech, Inc.
South San Francisco, CA

***Details of Genentech Career History***

2003 - 2005: Assistant then Associate Medical Director, Tarceva team, GNE
2005: Associate Medical Director, Exploratory Clinical Development, GNE
2005 - 2007: Medical Director then Senior Medical Director then Associate Group Medical Director; Avastin Breast Team
2007 - 2008: Associate Group Medical Director: T-DM1 team and Development Subteam Lead for T-DM1

2002 – 2003 Medical Director, Clinical Development
Chiron Corporation
Emeryville, California

1998 - 2002 Assistant Clinical Professor of Medicine
University of California at San Francisco
Division of Hematology/Oncology, Department of Medicine

1995 - 1998 Clinical Instructor
University of California at San Francisco
Division of Hematology/Oncology, Department of Medicine

## *Education*

1993 - 1995 University of California at San Francisco
Medical Oncology Fellowship



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| | |
|---|---|
| 1990 - 1993 | University of California at San Francisco<br>Internship and Residency<br>Internal Medicine, Categorical Program |
| 1986 - 1990 | University of California at Davis School of Medicine<br>Degree: M.D. |
| 1984 - 1985 | University of California at Davis |
| 1975 – 1979 | Indiana University at Bloomington<br>Degree: BS |

## *Honors and Awards*

| | |
|---|---|
| 1989 | Alpha Omega Alpha |
| 1986 – 1990 | Co-Class President, University of California Davis, School of Medicine |
| 1990 | Graduation Awards, University of California Davis, School of Medicine<br>• Department of Medicine Award<br>• American Medical Women's Association Award<br>• Lange Publication Award |
| 1995 | American Federation of Clinical Research<br>Excellence in Clinical Research Award |

## *Board Certifications, Licensure, Professional Society Memberships*

| | |
|---|---|
| 1993 | Internal Medicine |
| 1995 | Medical Oncology |
| G74133 | California State Medical License; issued May 26, 1992 |
| Member: | ASCO since 1995<br>AACR since 2001<br>ASH since 2002 |

## *Committee efforts, Teaching, Public Service*

| | |
|---|---|
| 2002 – 2002 | Chair, HPV Working Group<br>AIDS Malignancies Clinical Trials Consortium (AMC) |
| 1999 – 2001 | Co- Chair, HPV Working Group, AMC |
| 1999 – 2002 | Steering Committee, AMC |
| 1998 – 2002 | Kaposi's Sarcoma Working Group, Member, AMC |
| 1997 – 2002 | Director, Oncology Educational Electives for Residents, UCSF |
| 1999 – 2002 | UCSF Hematology / Oncology Fellowship Evaluation Committee, Member |



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| 1999- 2002 | Protocol Review Committee, UCSF Cancer Center, Member |
| 1997 – 1998 | Dean's Advisory Committee for AIDS, UCSF, Member |
| 1999 – 2002 | Advisory Committee to the UCSF AIDS Research Institute, Member |
| 1999 - 2000 | Patient / Physician Communication Task Force<br>National Comprehensive Cancer Network |

## *Publications*

*Manuscripts*


1. W. K. Alfred Yung, James J. Vredenburgh, Timothy F. Cloughesy, Phioanh (Leia) Nghiemphu, **Barbara Klencke**, Mark R. Gilbert, David A. Reardon, Michael D. Prados. Safety and Efficacy of Erlotinib in First-Relapse Glioblastoma: A Phase II Open-Label Study. Submitted (Oct 2008) for publication to NeuroOncology.
2. Chan AT. Tao Q. Robertson KD. Flinn IW. Mann RB. **Klencke B**. Kwan WH. Leung TW. Johnson PJ. Ambinder RF. Azacitidine induces demethylation of the Epstein-Barr virus genome in tumors. Journal of Clinical Oncology. 22(8):1373-81, 2004 Apr 15
3. **Klencke B**, Palefsky J. Anal Cancer: An HIV-related malignancy. Hematology and Oncology Clinics of North America. 17(3):859-72, 2003 Jun.
4. Rose B, Matthay KK, Price D, Huberty J, Klencke B, Norton JA, Fitzgerald PA. High dose $^{131}$I-Metaiodobenzylguanadine (MIGB) Therapy for 12 Patients with Malignant Pheochromocytoma. Cancer 2003; 98(2): 239-248.
5. Rodrigues L, **Klencke B**, Vin-Christian K, Berger TG, Crawford RI, Miller JR 3rd, Ferreira CMM, Nosrati M, and Kashani-Sabet M. Altered Clinical Course of Malignant Melanoma in HIV-Positive Patients. Archives of Dermatology 2002; 138:765-770.
6. **Klencke B**, Urban RG, Berry M, Matijevic, M, Lathey JL Thatcher J, Wilson J, Jay N, Palefsky J. Microsphere Encapsulated Plasmid DNA (pDNA) Treatment for HPV-16 Associated Anal Dysplasia: A Phase I Study of ZYC101. Clin Cancer Res 2002 8: 1028-1037.
7. Benson III AB, Mitchell E, Abramson N, **Klencke B**, Ritch P, Burnham JP, McGuirt C, Bonny T, Levin J, Hohneker J. Oral eniluracil/fluorouracil in patients with inoperable hepatocellular carcinoma. Ann Oncol. 2002 Apr;13(4):576-81.
8. Blackbourn DJ, Lennette E, **Klencke B**, Moses A, Chandran B, Weinstein M, Glogau RG, Witte MH, Way DL, Kutzkey, Herndier D, and Levy JA. The Restricted Cellular Host Range Of HHV-8. AIDS 2000; 14(9):1123-1133.
9. Hoffman R, Krieg R, **Klencke B**, Weinberg V, Welton ML. The significance of pretreatment CD4 count on the outcome and treatment tolerance of HIV-positive patients with anal cancer. Int. J. of Radiation Oncology Biol., Phys. 1999; 44(1):127-31.
10. **Klencke** B, Kaplan L. Advances and future challenges in Hon-Hodgkin's lymphoma. Current Opinion in Oncology 1998; 10: 422-427.
11. Dorsey BV, Benjamin LE, Rausher F, **Klencke B**, Venook AP, Warren RS, Weidner N. Intra-Abdominal Desmoplastic Small Round-Cell Tumor: Expansion of the Pathologic Profile. Modern Pathology 1996; 9 (6):703-709.




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*Abstracts*

- Brooks DJ, Cox J, Berman BS, Amare M, Benson AB, Masuoka L, **Klencke B**. Phase 2 study of tezacitabine in subjects with advanced/recurrent colorectal cancer. PASCO 2003, abstract 1122.
- **Klencke B**. HIV-Associated Anal Cancer: Biology, Epidemiology, Prevention and Treatment. PASCO, Educational Session, May 2002.
- **Klencke B**, Urban RG, Berry M, Thatcher J, Wilson J, Matijevic, M, Lathey JL, Jay N, Palefsky J. Results from a Phase I Trial of a Microsphere Encapsulated DNA Vaccine (ZYC101) for High-grade Anal Dysplasia. Oral Presentation. Fifth International AIDS Malignancies Conference, April 23-25, 2001.
- **Klencke B**. HPV in Anal and Penile Cancers. Papilloma virus: 18th International Conference. Barcelona July 21-28, 2000. Oral Presentation
- **Klencke B**, Berry JM, Thatcher J, Darraugh T, Rokovich J, Urban R, Palefsky J. Phase I Clinical Trial of HPV-16 E7 Vaccine for High Grade Anal Dysplasia. WHO / Eurogin Human Papillomavirus, Genital infections and Neoplasia Conference. Paris April 5-9, 2000. Oral Presentation 4/9/2000.
- Martin, J., Lee, T., Busch, M., Cheng, A., Graves, A., **Klencke, B.**, Cossen, C., Forghani, B., Osmond, D. Salivary Shedding Of KSHV Among Homosexual Men. Third Annual International HHV-8 Meeting. July 2000.
- Kaplan LD, Herndier B, **Klencke** B, Gascon R, Kesterson J, McGrath MS. Poyamine Analogue Therapy with Diethylhomospermine (DEHOP) for HIV-Associated non-Hodgkin's Lymphoma: Targeting Proliferating Macrophages. VII International Conference on Malignant Lymphoma 2-5 June, 1999, Lugano Switzerland Abstract # 105 p 35
- Kaplan LK, Hernier B, **Klencke** B, Gascon R, Kesterson R, McGrath MS. Diethylhomospermine (DEHOP) for HIV-associated non-Hodgkin's lymphoma (HIV-NHL). Third National AIDS Malignancies Conference May 26-27 1999, Abst 117.
- Benson AB, Mitchell E, Abramson N, **Klencke** B, Ritch P, Burnham J, McGuirt C, Levin J, Hohneker J. A multicenter, phase II trial of oral eniluracil plus 5-FU in patients with inoperable hepatocellular carcinoma. PASCO 1999, Abstract 985.
- Chang JWC, Vaquerano JE, Cadbury P, Peng M, Zhou YM, Wong B, Kashani-Sabet M, **Klencke** BJ, Sagabiel RW, Ituarte P, Leong SPL. Active specific immunotherapy with autologous tumor vaccine plus recombinant GMCSF with and without interferon alfa-2B mediates tumor regression in stage IV melanoma patients. PASCO 1999, Abstract 1697.
- Blackbourn D., Lennette E., Witte M., **Klencke** B., Levy J. Cellular host range of human herpesvirus 8 (HHV8). Abstract, AACR meeting 1997.
- **Klencke** B, Soetikno R, Corkins S, Venook AP, Warren RS, Garber A. Hepatic Artery Infusion Pump Therapy for Colorectal Cancer: Is It Cost-Effective? Abstract, AFCR Clinical Research Meeting 1995

*Chapters*

- **Klencke** B., Volberding P. AIDS-Related Malignancies. In: Holmes et al. Sexually Transmitted Diseases, 3rd edition. New York: McGraw-Hill, 1998, Chapter 76.



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# EXHIBIT B

Oncogene (1990), 5, 953–962

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# Expression of the HER-2/*neu* proto-oncogene in normal human adult and fetal tissues

Michael F. Press[1], Carlos Cordon-Cardo[2] and Dennis J. Slamon[3]

[1]*Department of Pathology, University of Southern California, 2011 Zonal Avenue, Los Angeles, California 90033;* [2]*Department of Pathology, Memorial Sloan-Kettering Cancer Center, 1275 York Avenue, New York, New York 10021;* [3]*Division of Hematology and Oncology, Department of Medicine, UCLA Medical School, Los Angeles, California 90024, USA*

**The HER-2/*neu* proto-oncogene is homologous with, but distinct from, the epidermal growth factor receptor. Current evidence indicates that this gene is frequently amplified and/or overexpressed in some human breast and ovarian cancers and that these alterations may be clinically important; however, little is known about the expression pattern of the gene in normal tissues. Using immunohistochemistry and northern blot analyses to identify the HER-2/*neu* protein and transcript respectively, we have evaluated a variety of normal adult and fetal tissues for HER-2/*neu* expression. HER-2/*neu* protein was identified on cell membranes of epithelial cells in the gastro-intestinal, respiratory, reproductive, and urinary tract as well as in the skin, breast and placenta. Northern hybridization confirmed the presence of the 4.5 kb transcript encoding the protein in these tissues. The amount of HER-2/*neu* message and protein was generally higher in fetal tissues than in the corresponding normal adult tissues. HER-2/*neu* expression levels in these normal tissues were similar to the levels found in non-amplified, non-overexpressing breast cancers and breast cancer cell lines. Southern hybridization of extracted DNA showed that none of the normal tissues expressing HER-2/*neu* had amplification of the gene. These results confirm that HER-2/*neu* is normally a membrane constituent of a variety of epithelial cell types.**

## Introduction

The *neu* oncogene was first isolated from DNA extracted from ethylnitrosourea-induced adrenal neuroglioblastomas of neonatal rats (Shih *et al.*, 1981). The oncogene was identified by its ability to function as a dominant transforming gene in NIH3T3 transfection assays. Since this gene was identified in DNA from neuroglioblastomas it was referred to as *neu* (Schechter *et al.*, 1984). Subsequently, three different groups independently identified the human homolog of this gene (Coussens *et al.*, 1985; Semba *et al.*, 1985; King, Kraus & Aaronson, 1985) and, because of homology to the human epidermal growth factor receptor (HER) and c-*erb*-B proto-oncogene, it was referred to as HER-2 (Coussens *et al.*, 1985) or c-*erb*B-2 (Semba *et al.*, 1985). Recently, amplification and overexpression of the HER-2/*neu* gene was found to be associated with shortened disease-free and overall survival in women with node-positive breast cancer (Slamon *et al.*, 1987; 1989a, b). In addition, an association between overexpression and clinical outcome has been seen in some women with node-negative breast cancer (Wright *et al.*, 1989; Ro *et al.*, 1989; Paik *et al.*, 1990). HER-2/*neu* amplification and overexpression have also been found in human ovarian carcinomas where the alteration is again associated with a shorter overall survival (Slamon *et al.*, 1989a, b). Amplification of this gene is consistently associated with overexpression of both the 4.5 kb messenger RNA encoding the gene and the p185 protein product. Several lines of experimental evidence indicate that alterations in HER-2/*neu* can play an important role in the pathogenesis of some animal tumors. Transgenic mice containing a mutated *neu* transgene driven by the mouse mammary tumor virus promoter consistently develop breast carcinoma (Muller *et al.*, 1988). Transfection and subsequent amplification/overexpression or overexpression alone of a normal HER-2/*neu* gene (Hudziak *et al.*, 1987; diFiore *et al.*, 1987) renders NIH3T3 cells more tumorigenic in nude mice. The HER-2/*neu* gene encodes a membrane protein with an extracellular domain, which makes it a candidate target for immunotherapeutic approaches against those cells expressing high levels of gene product. Experimental data using *neu*-transformed cell lines grown *in vitro* as well as in nude mice suggest that this type of approach may be feasible (Drebin *et al.*, 1985). To exploit these potential therapies in patients it is important to understand the distribution and relative expression levels of HER-2/*neu* in normal tissues. Little information is currently available regarding this issue. This study describes the distribution of HER-2/*neu* expression in normal adult and fetal tissues and characterizes the expression levels.

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## Results

HER-2/*neu* expression, in both fetal and adult tissues, was identified immunohistochemically on the membranes of epithelial cells (Table 1, Figures 1 and 2). This staining pattern was found throughout the gastro-intestinal, respiratory, urinary, and reproductive tracts, as well as the skin of fetal and adult specimens. Levels of expression were, in almost all instances, higher in fetal than in the corresponding adult tissues. Immunostaining of frozen, normal tissues ranged from barely detectable to, in a few specimens, moderate intensity (2+) (Table 1). Immunostaining in formalin-fixed, paraffin-embedded tissues was also seen on cell membranes; however, these sections consistently showed less immunostaining than the corresponding frozen sections and, frequently, immunostaining detectable in frozen sections was completely absent in paraffin-embedded sections of the same specimen. The same phenomenon has been previously described for this protein in human tumor tissue (Slamon *et al.*, 1989a, b). Because of this

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Exhibit 1029-15

**Table 1** Expression of HER-2/*neu* oncogene in fetal and adult tissues

| *Tissues* | *Immunostaining for HER-2/*neu* Fetal* | *Adult* |
|---|---|---|
| Breast | NE | + |
| *Female reproductive* | | |
| Ovary | – | –* |
| Fallopian tube | + | + |
| Uterus-endometrium | + | + |
| Uterus-cervix | + | wk/+ |
| Vagina | + | + |
| *Male reproductive* | | |
| Testis | – | – |
| Prostate | NE | wk/+ |
| Epididymus | NE | – |
| *Digestive* | | |
| Oropharynx | + | wk/+ |
| Salivary gland | NE | wk |
| Esophagus | + | wk |
| Stomach | + | wk |
| Small intestine | + | wk |
| Large intestine | + | wk |
| Liver-hepatocytes | – | – |
| Bile ducts | + | wk |
| Pancreatic ducts | + | wk |
| Pancreatic acini | – | – |
| *Urinary* | | |
| Kidney | +/++ | wk/+ |
| Ureter | + | wk |
| Bladder | + | –/wk |
| *Circulatory* | | |
| Heart | – | – |
| Arteries | – | – |
| Veins | – | – |
| Capillaries | – | – |
| *Respiratory* | | |
| Bronchi | + | + |
| Alveoli | 0 | – |
| *Hematologic* | | |
| Liver | – | – |
| Spleen | – | – |
| Bone marrow | – | – |
| Lymph node | NE | – |
| *Musculoskeletal* | | |
| Bone | – | – |
| Cartilage | – | – |
| Muscle | – | – |
| *Skin* | | |
| Epidermis | + | + |
| Adnexae | 0 | wk |
| *Endocrine* | | |
| Pituitary | – | – |
| Adrenal cortex | – | – |
| Adrenal medulla | – | – |
| Pancreatic islets | – | – |
| *Central Nervous System* | | |
| Forebrain (cerebral cortex) | – | – |
| Midbrain | – | – |
| Hindbrain (cerebellum, medulla) | – | – |
| Spinal cord | – | – |
| Meninges | – | – |
| Eye | – | NE |

*Surface epithelium had weak membrane immunostaining, but the remainder of the ovary was not immunoreactive
NE = not examined

difference, immunohistochemical analyses for the current study were performed using frozen specimens, circumventing the possibility that a weak or negative result would be due to fixation or tissue processing artifacts. The data on expression of HER-2/*neu* in normal fetal and adult tissues are summarized in Table 1 and are discussed in detail below.

*Gastrointestinal tract*

HER-2/*neu* immunoreactive epithelium lined the fetal and neonatal gastrointestinal tract from the oropharynx to the large intestine. The epithelial cells of the common bile duct and pancreatic ducts also showed HER-2/*neu* membrane immunoreactivity. Membrane staining of epithelial cells ranged from weakly positive to distinctly positive (Figure 2P, Q). The lateral and basal cell membranes of most gastrointestinal epithelia were immunostained for HER-2/*neu*. Subsequent to the first trimester, the small and large intestine were easily distinguishable and it was apparent that the apical cell membranes of intestinal epithelial cells were negative or only weakly immunostained for HER-2/*neu*. Cells of the lamina propria, muscularis and serosa showed no immunoreactivity.

The epithelium of the adult gastrointestinal tract showed membrane immunoreactivity for HER-2/*neu*; however, the amount of immunostaining was generally reduced below the level observed in fetal tissue (Figure 2, Q vs. R) and in some cases, was barely detectable. Stratified squamous epithelium of the oropharynx and esophagus was weakly immunostained. Ductal epithelium of salivary glands was also immunoreactive. The columnar epithelium of the gastric pits was weakly immunostained while the epithelial cells of the gastric glands (parietal cells, chief cells) showed no HER-2/*neu* immunoreactivity. In the cardia and antrum the foveolae, which are lined almost entirely by mucus-secreting epithelial cells, showed weak immunostaining for HER-2/*neu* throughout their depth. Similarly, both the small intestinal epithelium (Figure 2) and the colonic epithelium were weakly immunoreactive for HER-2/*neu*. The immunostaining was again found along the lateral and basal cell membranes of these epithelial cells. In the small intestine, the epithelial cells lining the villi showed slightly more immunoreactivity than the epithelial cells of the crypts. Epithelial cells of Brunner's glands also had HER-2/*neu* membrane immunostaining. As in the fetal tissue, none of the cells of the lamina propria, muscularis mucosa, submucosa, muscularis propria or serosa showed HER-2/*neu* immunostaining. Membranes of ductal epithelial cells in the pancreas were weakly immunostained but acini and islet cells were not. Intrahepatic bile ducts were the only cell types in the adult liver which had detectable membrane staining (Figure 2L, M). This membrane staining was very weak even in larger bile ducts and often not identified in smaller bile ducts. Epithelial cells of the gall bladder also showed membrane immunostaining.

*Respiratory tract*

The epithelium of the developing lungs (presumptive trachea, bronchi, and bronchioles) was immunoreactive for HER-2/*neu* in all stages of fetal development and in the neonate (Figure 2E, F), with the exception of the 0.3 cm fetus where the lung buds were not identified. Mesenchymal and vascular cells were negative for HER-2/*neu*.

Adult lungs showed HER-2/*neu* low level immunoreactivity on bronchial and bronchiolar epithelial cell membranes (Figure 2G, H), especially the lateral and basal portions of the cell membranes. The alveoli were

PHIGENIX
Exhibit 1029-16

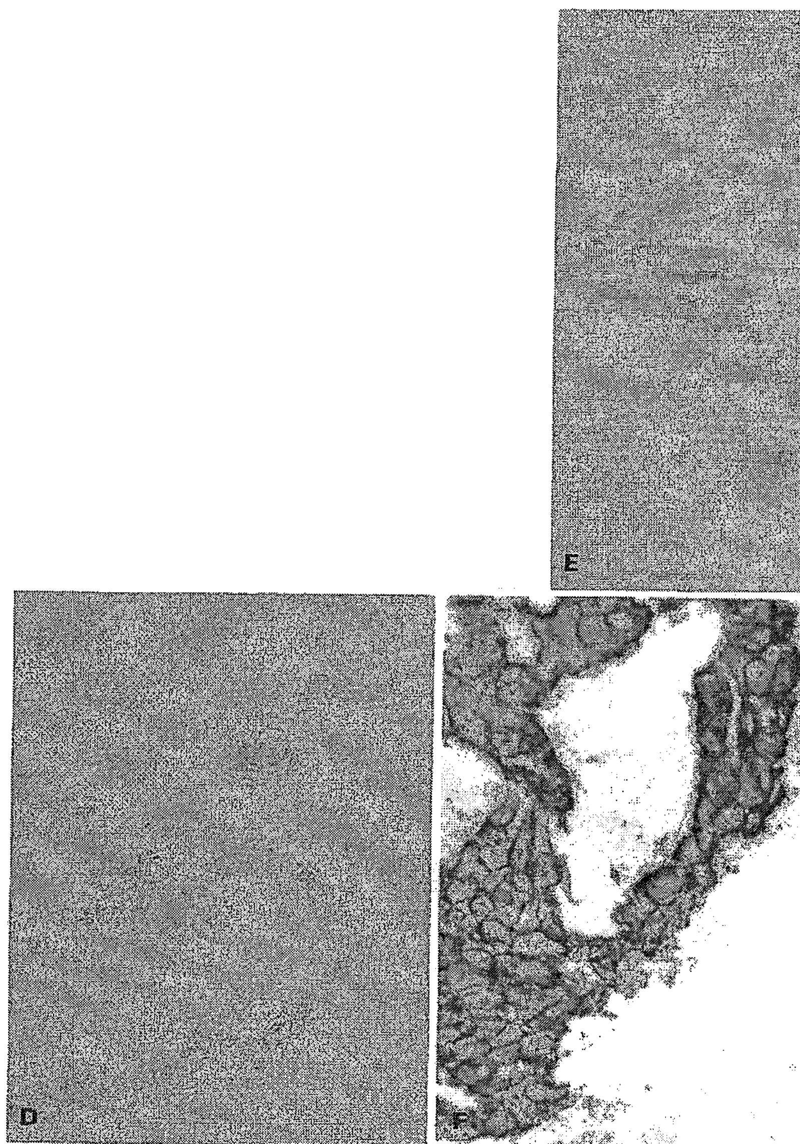


**Figure 1** Normal adult breast. All normal breast specimens had at least weak membrane immunostaining in frozen tissue sections. This illustrative case, obtained at reduction mammoplasty, had the strongest HER-2/*neu* membrane immunostaining (2+) in our series of 21 normal breast samples. (**A**) Hermatoxylin and eosin stained section showing normal breast lobules and ducts. Magnification, 150×. (**B**) Immunohistochemical localization of HER-2/*neu* demonstrates immunostaining throughout the ductal and lobular epithelial cells in a frozen tissue section. Magnification, 150×. (**C**) Immunostaining of epithelial cell membranes in a terminal duct lobular unit. Frozen tissue section. Magnification, 1450×. (**D**) Negative control section in which pre-immune serum was used instead of HER-2/*neu*-immune serum. Frozen tissue section. Magnification, 1450×. (**E**) A portion of the same specimen, fixed in formalin and processed for paraffin-embedding, was sectioned and processed for HER-2/*neu* localization as in **C**. Note the relative lack of immunostaining. Magnification, 1450×. (**F**) A breast carcinoma known to have 5 to 20-fold amplification and overexpression of the HER-2/*neu* gene demonstrates strong (3+) membrane staining for comparison with **C** and **E**. Frozen tissue section. Magnification, 1450×

not immunoreactive nor were pulmonary blood vessels, lymphatics or connective tissue.

*Urinary tract*

Mesonephric and metanephric epithelium was immunostained for HER-2/*neu* in all fetuses where the kidneys were examined (nine cases) as well as in the neonate. Renal epithelium of the developing kidney, i.e., epithelium lining the parietal surface of Bowman's capsule (Figure 2S), the proximal tubule, loops of Henle, distal tubule and collecting tubule (Figure 2T), expressed HER-2/*neu* (Figure 2). The intensity of the immunostaining ranged from weak in Bowman's capsule and the proximal tubules to moderate (2+) immunostaining in the collecting tubules of the kidney. Urothelium of the renal pelvis and developing bladder also expressed the protein.

In the adult kidney the distribution of immunostaining is similar to that observed in the fetuses, but the intensity was much weaker in the glomeruli, proximal and distal tubules. The collecting tubules were more strongly immunostained (+/++) (Figure 2U, V) than the glomeruli and tubules of the adult kidney. The urothelium of the ureter and bladder were only weakly immunoreactive.

*Female reproductive tract*

Epithelial cells throughout the developing mullerian ducts of the embryos and fetuses were immunostained

PHIGENIX
Exhibit 1029-17

Fetus

Adult

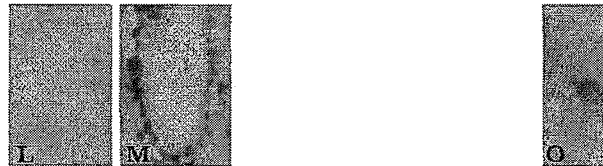


**Figure 2** Immunohistochemical identification of HER-2/*neu* in normal fetal and adult tissues. The left half of the figure is devoted to illustrating the results obtained in fetal tissues. The right half illustrates the results obtained in the corresponding adult tissues. All of the results illustrated were obtained with frozen tissue sections. (**A**) Histologic section of fetal brain (developing cerebral cortex) stained with hematoxylin and eosin corresponding to the immunostained area shown in **B**. 725×. (**B**) Fetal brain (developing cerebral cortex) incubated with HER-2/*neu* immune serum (#60) as part of the peroxidase antiperoxidase technique shows no immunostaining. No counterstain. 725×. (**C**) Histologic section of adult cerebral cortex stained with hematoxylin and eosin corresponding to **D**. (**D**) Adult cerebral cortex similarly shows no immunoreactivity with HER-2/*neu* immune serum. No counterstain. 725×. (**E**) Histologic section of a bronchus in fetal lung stained with hematoxylin and eosin corresponding to the area shown in **F**. 1450×. (**F**) Bronchial epithelium of fetal lung shows membrane staining for HER-2/*neu*. No counterstain. 1450×. (**G**) Adult bronchial epithelium also shows HER-2/*neu* membrane staining. No counterstain. 1450×. (**H**) Serial section of adult lung stained with hematoxylin and eosin showing the same bronchus at a lower magnification. 725×. (**I**) Histologic section of fetal liver stained with hematoxylin corresponding to area shown in **J**. 1450×. (**J**) Fetal liver shows no HER-2/*neu* immunostaining in hepatocytes or hematopoietic cells by immunohistochemistry. No counterstain. 1450×. (**K**) HER-2/*neu* immunoreactivity is identified in cell membranes of the fetal biliary epithelium. No counterstain. 1450×. (**L**) Weak HER-2/*neu* immunostaining is also identified on cell membranes of adult biliary epithelium. 1450×. No counterstain. (**M**) Histologic section of biliary epithelium stained with hematoxylin and eosin corresponding to the area shown in **L**. 1450×. (**N**) Immunoreactivity for HER-2/*neu* is not identified in hepatocytes of adult liver. No counterstain. 1450×. (**O**) Histologic section of normal adult liver stained with hematoxylin and cosin corresponding to area shown in **N**. 1450×. (**P**) Histologic section of fetal small intestine stained with hematoxylin and eosin corresponding to

PHIGENIX
Exhibit 1029-18

for HER-2/*neu*. The various components of the female reproductive tract (vagina, cervix, uterus and fallopian tube) could be identified in fetuses larger than 10 cm and all were immunoreactive for HER-2/*neu* (Figure 2W, X, AA, BB). As in the gastro-intestinal tract, the protein was localized to the lateral and basal cell membranes of these cells. The apical portion of the cuboidal and columnar epithelial cells (microvillus brush border) usually lacked HER-2/*neu* immunostaining (Figure 2X). Stromal cells, smooth muscle cells, blood vessels and mesothelial cells also lacked immunoreactivity for HER-2/*neu*. Finally, the developing ovaries from fetuses were not immunostained for HER-2/*neu*.

The adult vaginal epithelium was immunoreactive for the protein throughout its entire thickness including basal cells, parabasal cells, intermediate cells and superficial cells. The superficial cells showed variable immunostaining ranging from not present to weak. Immunostaining was strongest in the parabasal and intermediate cells. This immunostaining pattern was present in vaginal tissue sampled throughout the menstrual cycle. Vaginal samples obtained during menstruation or the early follicular phase were immunostained throughout the full thickness but displayed weaker staining. During the remainder of the menstrual cycle the immunostaining was slightly stronger. The submucosa and muscularis of the vagina were not immunoreactive for HER-2/*neu*.

Immunostaining of the adult cervix was identified in the ectocervical squamous and endocervical columnar epithelia. Stromal cells, smooth muscle cells of the muscularis and cells of blood vessels did not express HER-2/*neu*.

HER-2/*neu* immunoreactivity was present in the adult uterine epithelium (Figure 2Y, Z). Endometrial surface and glandular epithelia were stained throughout the menstrual cycle and again the immunoreactivity was identified predominantly along the lateral and basal aspects of cell membranes. Stromal cells of the endometrium were HER-2/*neu* negative during the normal menstrual cycle and after menopause. However, during the first and second trimesters of gestation some decidualized stromal cells did express the protein. Glandular epithelial cells, especially hypersecretory glands of the gravid uterus showed stronger HER-2/*neu* immunostaining than during the menstrual cycle. This increased membrane immunostaining was most apparent along the basal aspects of cell membranes. Decidualized stromal cells identified on the maternal surface of the placenta at delivery also showed membrane immunoreactivity.

The adult fallopian tube showed weak to moderate immunostaining of tubal epithelial cells throughout the menstrual cycle (Figure 2 CC, DD). Immunostaining was less intense during menstruation and the early proliferative phase. Stromal cells, blood vessels, smooth muscle cells of the muscularis and mesothelial cells of the serosa were not immunoreactive.

With the exception of the surface epithelial cells, the ovary was not immunoreactive for HER-2/*neu*. Weak immunostaining of surface epithelium was identified in two of three ovaries where the epithelium had not become detached; one ovary was removed from a post-menopausal woman and the other from a woman of reproductive age during the luteal phase of the menstrual cycle.

*Breast*

Breast tissue was not identified in any of the embryos or fetuses. Adult breast consistently showed weak to low levels of HER-2/*neu* immunoreactivity in both ductal and lobular epithelia. The basal and lateral cell membranes were immunostained at levels comparable to those observed in most fetal epithelial cells. Immunostaining did not vary significantly during the menstrual cycle when 21 separate specimens distributed equally throughout the cycle were examined. In general, the amount of immunostaining in normal breast tissue was similar to that associated with non-HER-2/*neu*-amplified breast cancers, i.e. those which have a single copy of the gene and low levels of expression (Slamon *et al.*, 1989a, b). Of the 21 normal breast specimens evaluated, only one showed moderate levels (++) of staining (Figure 1b and c); however, this level was still lower than that observed in most amplified/overexpressing tumors (Figure 1f). The difference in HER-2/*neu* staining intensity seen in frozen (Figure 1b and c) and formalin-fixed, paraffin-embedded sections (Figure 1e) of the same specimen can be readily appreciated in this case. Other cells in the normal breast including myoepithelial cells, fibroblasts, fat cells, vascular smooth muscle and endothelial cells were not immunostained for HER-2/*neu*.

*Male reproductive tract*

Glandular epithelium of the adult prostate was weakly immunostained for HER-2/*neu*, while the stromal and vascular cells of the gland were negative. Fetal and adult testis as well as adult epididymus were not HER-2/*neu* immunoreactive.

*Placenta*

The squamous epithelium covering the fetal surface of the placenta and umbilical cord had immunoreactive

the area shown in **Q**. 1450×. **(Q)** HER-2/*neu* immunostaining of fetal small intestinal epithelial cells. No counterstain. 1450×. **(R)** Immunolocalization of HER-2/*neu* to the cell membranes of epithelial cells of the adult duodenum. No counterstain. 1450×. **(S)** Immunohistochemical localization of HER-2/*neu* in the metanephros showing immunostaining of cells lining Bowman's capsule of a glomerulus (g) and a cortical tubule. No counterstain. 1450×. **(T)** The developing collecting tubules in the medulla of the metanephros show membrane immunostaining of epithelial cells. No counterstain. 1450×. **(U)** Tubular epithelium of the adult medulla also shows membrane immunostaining for HER-2/*neu*. No counterstain. 1450×. **(V)** Histologic section of renal medulla corresponding to U. 1450×. **(W)** Histologic section of fetal endometrium stained with hematoxylin and eosin showing the same area as **X**. 1450×. **(X)** HER-2/*neu* is localized to the cell membranes, especially the lateral and basal cell membranes, of endometrial epithelial cells by immunohistochemistry in this fetal uterus. No counterstain. 2900×. **(Y)** The distribution of HER-2/*neu* in the adult endometrium is limited to the membranes of the epithelial cells. No counterstain. 1450×. **(Z)**. Histologic section of **Y** stained with hematoxylin and eosin. 1450×. **(AA)** Histologic section of fetal fallopian tube stained with hematoxylin and eosin corresponding to area shown in **BB**. 1450×. **(BB)** Immunohistochemical localization of HER-2/*neu* in fetal fallopian tube. The immunostaining is present on cell membranes of epithelial cells. No counterstain. 1450×. **(CC)** HER-2/*neu* is also localized to the cell membranes of epithelial cells in the adult fallopian tube. No counterstain. 1450×. **(DD)** Histologic section of **CC** stained with hematoxylin and eosin. 1450×

PHIGENIX
Exhibit 1029-19

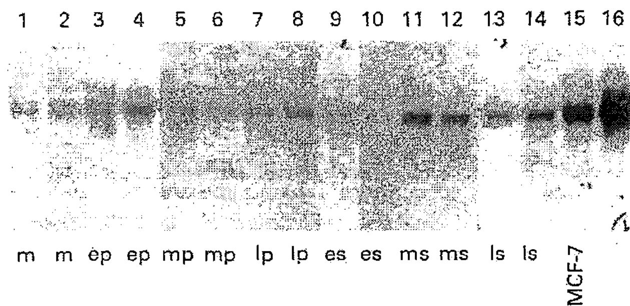


**Figure 3** (A) Northern blot analysis of HER-2/*neu* expression in normal fetal and adult tissues. The HER-2/*neu* message is identified as an autoradiographic signal in lanes 1 through 8 of this Northern blot. Only lane 1 (SKBR3, control breast cancer cell line with amplification and overexpression of the gene) has levels of expression significantly above the level found in a single-copy, low expression breast cancer shown in lane 2 (MCF-7 human breast cancer cell line). Expression of HER-2/*neu* message is identified in normal fetal and adult kidney (lane 3 and lane 4, fk and ak), fetal and adult lung (lanes 5 and 6, fl and al) and fetal and adult intestine (lanes 7 and 8, fi and ai) at levels similar to that in low expression breast cancers (e.g. lane 2). The 4.5 kb transcript is not identified in total RNA from fetal liver (fh, lane 9), adult liver (ah, lane 10), fetal cerebral cortex (fb, lane 11), adult cerebral cortex (ab, lane 12) and adult spleen (as, lane 13). In addition, to confirm the apparent lack of HER-2/*neu* expression in liver, 10, 20, 30 and 40 micrograms of total RNA from adult liver were loaded in lanes 14, 15, 16 and 17, respectively. Autoradiograms were produced by exposing the filter for 72 h at −70°C. Exposure of the filter for an additional 48 h did not show an increase in HER-2/*neu* transcript. The Northern blots were prepared with total RNA from each sample as follows: 10 micrograms of total RNA were loaded in lanes 1–9 and 11–14; 20 micrograms in lanes 10 and 15; 30 micrograms in lane 16 and 40 micrograms in lane 17. (B) Controls–28S ribosomal RNA. To confirm that the loading and transfer of RNA had been performed properly the filters were stained with ethidium bromide to demonstrate the 28S and 18S subunits of ribosomal RNA. Staining of the 28S subunit is illustrated

**Figure 4** Northern blot analysis of HER-2/*neu* expression in normal adult endometrium throughout the menstrual cycle. HER-2/*neu* message (4.5 kb) is identified in the RNA from two menstrual (m-lanes 1, 2), two early proliferative phase (ep-lanes 3, 4), two midproliferative phase (mp-lanes, 5, 6), two late proliferative phase (lp-lanes 7, 8), two early secretory phase (es-lanes 9, 10), two midsecretory phase (ms-lanes 11, 12) and two late secretory phase (ls-lanes 13, 14) endometria. The low level of expression present in normal endometrium is clearly demonstrated by comparison of the autoradiographic signals obtained from endometrial samples (lanes 1–14) with the single copy, low HER-2/*neu* expressing breast cancer cell line, MCF-7 (lane 15). Lane 16 contains a breast cancer with 2 to 5-fold amplification of the HER-2/*neu* gene and moderate overexpression of the HER-2/*neu* message. The Northern blot was prepared as in Figure 3. Endometrial samples were dated from formalin-fixed, paraffin-embedded, hematoxylin and eosin stained tissue sections according to morphologic criteria described elsewhere (Press *et al.*, 1984; Press *et al.*, 1986; Press, Udove & Greene, 1988)

cell membranes during gestation and at delivery. The epithelium of the allantoic remnant in the umbilical cord was also immunostained for HER-2/*neu*. Trophoblast of the chorionic villi expressed HER-2/*neu* during the first and second trimesters but only weakly or not at all at delivery. Interestingly, the immunostaining for HER-2/*neu* was along the apical cell membrane of the syncytial trophoblast. Some maternal decidualized stromal cells of gestational endometria were immunoreactive for HER-2/*neu* during the first and second trimester and at term and expressed the protein circumferentially along the cell membrane.

*Skin*

Skin of the embryos was composed of only a few layers of squamous epithelium and these cells expressed HER-2/*neu*. Adult skin showed immunoreactivity of keratinocytes but not melanocytes. Adnexal structures, especially hair follicles, sweat glands and sebaceous glands, were weakly immunostained.

*Other tissues*

The fetal and adult central nervous system was not immunoreactive for HER-2/*neu* (Table 1, Figure 2A–D). The developing eye, cerebral cortex, infundibulum, thalamus, pons, medulla oblongata showed no membrane immunostaining. Likewise, the mantle, marginal zones and dorsal root ganglia of the developing spinal cord showed no protein staining. Only epithelial cells of the choroid plexus showed weak immunoreactivity. Although cartilage did not express HER-2/*neu*, notochord of developing vertebral cartilage in embryos was immunoreactive for the protein. Hematopoietic tissues including fetal liver (Figure 2I, J) spleen and adult bone marrow did not have HER-2/*neu* staining. In the adult liver only bile ducts showed membrane staining which was weak and inconsistently present (Figure 2L, M). Hepatocytes of adult liver were not immunostained (Figure 2N, O). The red and white pulp of adult spleen, adult lymph nodes and thymus, and lymphoid cells of adult tonsils were also not immunoreactive; however, the tonsillar squamous epithelium had membrane immunostaining. The fetal heart, adrenal gland (cortex and medulla), islet of Langerhans were not immunostained for HER-2/*neu*.

*Expression of HER-2/*neu* characterized by Northern hybridization*

Northern blot hybridization analyses were used for independent confirmation of HER-2/*neu* expression in fetal and adult tissues. Tissues which were immunostained by HER-2/*neu* antiserum had the 4.5 kb HER-2/*neu* message providing independent confirmation of expression (Figure 3, lanes 3–8). The MCF-7 human breast cancer cell line is an example of single DNA copy, low expression of HER-2/*neu* (Figure 3, lane 2; Figure 4, lane 15). The level of RNA expression in all normal fetal and adult tissues was similar to that found in single copy, low expressing breast cancers (Figure 3). RNA expression in the fetal kidney was higher than in other normal tissues. Fetal and adult liver, fetal and adult brain, fetal heart and adult spleen (Figure 3, lanes 9–14) did not express detectable levels of the HER-2/*neu* message, confirming the immunohistochemical data.

To determine if HER-2/*neu* expression levels varied with the menstrual cycle, we analyzed total RNA from 32 normal endometria including; 4 early proliferative phase, 4 midproliferative phase, 4 late proliferative phase, 4 early secretory phase, 4 midsecretory phase, 4 late secretory phase, 4 menstrual endometria and 4 gestational endometria. The amount of HER-2/*neu*

PHIGENIX
Exhibit 1029-20

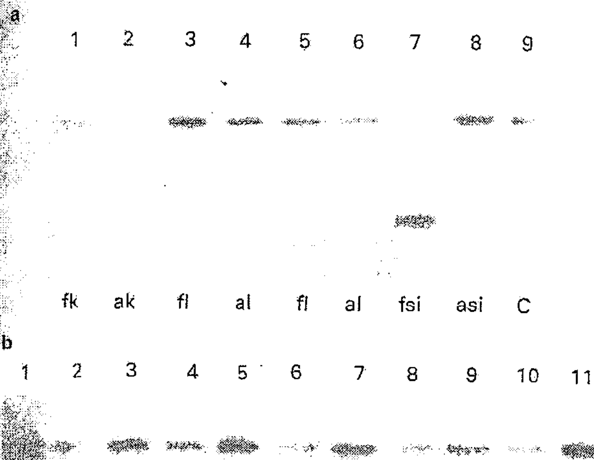


**Figure 5** Southern blot analysis of HER-2/*neu* gene copy number in normal adult and fetal tissues. (**a**) Comparison of selected fetal and adult normal tissues. A single copy of the HER-2/*neu* gene is present in each of fetal and adult kidney (lanes 1 and 2, fk and ak), fetal and adult lung (lanes 3, 5 and 4, 6; fl and al) and fetal and adult small intestine (lanes 7 and 8; fsi and asi). A single copy breast cancer cell line, MCF-7, (lane 9, C) is also included as a control. The DNA from the fetal small intestine illustrated in lane 7 shows a rearrangement of the HER-2/*neu* gene and was the only case which showed this change. The fetal kidney DNA of lane 1 is from the same case showing overexpression in Figure 3, lane 3. (**b**) Normal adult endometrium from menstrual (lanes 1 and 2), early proliferative (lanes 3 and 4), midproliferative (lane 5), late proliferative (lanes 6 and 7), early secretory (lane 8), midsecretory (lane 9) and late secretory (lane 10) have a single copy of the HER-2/*neu* gene. DNA from a human breast cancer cell line (lane 11) is used as the single copy control

message identified in endometria at any time point during the menstrual cycle did not exceed that of single copy, low expressor breast cancer cells as illustrated in Figure 4 (lanes 1–14) again using MCF-7 as the low expressor control (Figure 4, lane 15). While there was slight variation in a few cases, there was no systematic change in expression during the menstrual cycle. Gestational endometria showed similar levels of HER-2/*neu* RNA expression. This is comparable to the results obtained by immunohistochemistry.

### *Structure of the HER-2/*neu *gene in normal tissues*

Southern hybridization was performed to determine if any of the tissues expressing HER-2/*neu* had alterations of the gene, i.e. amplification or rearrangements. Southern hybridization of DNA from tissue derived from fetal and adult kidney, lung, colon, liver, spleen and adult endometrium showed no evidence of gene amplification or rearrangement, (Figure 5), with the exception of a small intestine from one fetus which did show an apparent rearrangement (Figure 5a, lane 7).

## Discussion

In this study HER-2/*neu* expression was analysed in a large variety of normal fetal and adult tissues. The cells expressing HER-2/*neu* were derived from all three germ layers. Ectodermal derivatives, i.e. skin and mammary glands, mesodermal derivatives, i.e. kidney, ureter, vagina, cervix, uterus, fallopian tube and Wolffian duct, and endodermal derivatives, i.e. oropharynx, esophagus, stomach, intestines, pancreas, biliary tree, lungs, prostate, and bladder expressed the HER-2/*neu* gene. Extraembryonic tissues expressing HER-2/*neu* were limited to the trophectoderm of chorionic villi. Endothelial cells and mesothelial cells were the only polarized cells attached to basement membrane which did not express HER-2/*neu*. Interestingly, these two cell types are not considered 'true' epithelia (Ekblom, 1989). The pattern of HER-2/*neu* expression emerging from this study is of weak levels of expression distributed in epithelial cells of most organs.

HER-2/*neu* expression in adult endometrium did not show cyclic variation with the menstrual cycle either by immunohistochemistry or by northern blot analysis. Likewise, immunohistochemical assay of normal adult breast also did not show cyclic variation. In another series of normal breasts, reported elsewhere, HER-2/*neu* expression was demonstrated with both northern hybridization and immunohistochemistry (Bartow *et al.*, 1989). The lack of cyclic variation indicates that HER-2/*neu* expression is not significantly altered by steroid hormones.

HER-2/*neu* was not preferentially expressed in dividing cells. Expression in secretory endometria was as great as, or slightly greater than, it was in proliferative endometria. Basal cells of squamous epithelia, columnar cells of crypts in the gastrointestinal tract, and cytotrophoblastic cells of placental chorionic villi, the proliferative compartment of their respective tissues, all showed similar or less immunostaining than adjacent epithelial cells.

The level of HER-2/*neu* expression in normal tissue, both by subjective evaluation of immunostaining results and by quantitation of northern hybridization, was consistently less than that observed in breast cancers with amplification and/or overexpression of the gene, and was similar to that observed in breast cancers lacking HER-2/*neu* amplification and overexpression. HER-2/*neu* protein product identified by immunohistochemistry was generally greater in fetal tissues than in their adult counterparts with the exception of the female reproductive tract and breast where it appeared to be comparable to fetal levels. Only the fetal metanephros (kidney) had levels of expression which overlapped with the low end of the spectrum of breast cancers showing overexpression of HER-2/*neu*. In contrast to mesothelium lining the peritoneal cavity, the surface epithelium of the ovaries was immunostained for HER-2/*neu*.

During the preparation of this manuscript four publications appeared reporting the distribution of HER-2/*neu* expression in normal human fetuses and/or adult tissues (Cohen *et al.*, 1989; DePotter *et al.*, 1989; Mori *et al.*, 1989; Quirke *et al.*, 1989). The distribution of HER-2/*neu* expression described here is in general agreement with only one of these reports (Mori *et al.*, 1989). Of these four publications only Mori *et al.* (1989) used frozen tissue for their study and only they described HER-2/*neu* as localized to cell membranes. Mori *et al.* (1989) reported their preliminary findings in three frozen human fetuses; 9, 14 and 24 weeks of age. They identified the protein on cell membranes of epithelial cells from a variety of organs (lung, esophagus,

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stomach, intestines, kidney, and pancreas). Conversely, Quirke *et al.* reported broad expression of HER-2/*neu* including 'staining' of neural processes in both the central and peripheral nervous systems, chondrocytes of developing cartilage, osteocytes in bone, rhabdomyoblasts, cardiac muscle, respiratory epithelium and mesenchyme/smooth muscle, liver, mesonephric glomeruli, tubules and ducts, metanephric glomeruli and tubules, renal pelvis epithelium, gonadal blastema, adrenal gland, pancreatic acini, epidermis of skin and cyto- and syncytiotrophoblast from 11 formalin-fixed, paraffin-embedded fetuses. The cellular and subcellular distribution of this staining was unclear, but was not described as solely distributed on cell membranes. 'Membranous staining' is described only once, in the liver of a 6 week fetus. 'Strong punctate staining' was identified in the mesonephric tubules and in adult renal tubules. 'Cytoplasmic staining' was present in chondrocytes and in osteocytes. 'Oesophageal, gastric, small intestinal and colonic epithelium stained diffusely but with luminal accentuation'.

Localization of the putative receptor protein, HER-2/*neu*, to cell membranes which we (Slamon *et al.*, 1989a, b) and others (Van de Vijer *et al.*, 1988) consider to be characteristic was apparently not identified in these formalin-fixed, paraffin-embedded fetuses (Quirk *et al.*, 1989). The two antisera used by Quirk *et al.* (1989) to identify HER-2/*neu* in fetuses have been previously characterized as giving 'qualitatively and quantitatively different staining reactions. In all of the positive tumors, antiserum 20N predominantly stained the cytoplasm with only one tumor exhibiting membrane staining. ... Antiserum 21N predominantly stained the cell membrane ...' (Gusterson *et al.*, 1988). In the more recent publication reporting immunostaining in normal tissue 'the pattern of staining of both antibodies was identical, except for the strong staining of developing and mature red cells by 21N' (Quirk *et al.*, 1989). However, it is unclear whether the pattern was of membrane or cytoplasmic immunostaining or both. One possibility is that variations may be a result of differences in sample preparation, i.e. fixative, fixation time, embedding temperatures, etc.

Others, using Bouins-fixed, paraffin-embedded tissues, described HER-2/*neu* immunoreactivity in the gastrointestinal tract but likewise did not describe immunostaining on cell membranes (Cohen *et al.*, 1989).

DePotter *et al.* (1989), also using paraffin-embedded tissues, found 'a constant diffuse intracytoplasmic granular staining' in a variety of normal adult and fetal tissues. However, 'the cytoplasmic reacting protein was shown to be a different protein from the known *neu* oncogene product of 185 kd'; it had a molecular weight of 155 kd (DePotter *et al.*, 1989). They suggest that 'it could be a *neu*-like cross-reacting protein or a different *neu* oncogene product, derived by alternative splicing from the same gene and having a different destination in the cell'. No data was provided to show a change in the signal region of the peptide nor was any data provided showing alterations in the transmembrane domain which would prevent the protein product from accumulating in the cell membrane. Western immunoblots have been used in selected normal tissues to confirm that HER-2/*neu* has the characteristic 185 kd size (Mori *et al.*, 1989). Our experience with formalin-fixed, paraffin-embedded normal tissues as well as with breast cancers (Slamon *et al.*, 1989a and b) is that HER-2/*neu*, when identified, is localized to cell membranes in this material. Samples with high levels of HER-2/*neu* amplification and marked overexpression can frequently have some cytoplasmic staining; however, we do not see this in the absence of accompanying membrane staining. Neither have we observed a redistribution of the HER-2/*neu* protein from the membrane to the cytoplasm with paraffin-embedding. The amount and intensity of the membrane immunostaining for HER-2/*neu* is consistently reduced when paraffin-embedded tissue is compared to frozen tissue sections derived from the same specimen (Slamon *et al.*, 1989a and b).

The assessment of the normal expression pattern of this gene is of some importance not only for general biologic consideration, but also because of potential utility of measurement of its expression in diagnostic and prognostic tests for human breast and ovarian cancer (Slamon *et al.*, 1989a). Moreover, therapeutic approaches directed at this protein will most likely be based on the differential high levels of expression seen in some tumors compared to normal tissues. As a result, knowledge of the amount and distribution of the HER-2/*neu* protein in normal tissues is of importance in interpreting diagnostic assays and could be of importance in anticipating toxicity of future therapeutic trials directed at the alteration in expression of this protein in some human tumors.

## Materials and methods

### *Embryonic/fetal tissues*

Both frozen and formalin-fixed, paraffin-embedded embryonic and fetal tissues were obtained from twelve human fetuses of between approximately 3 and 24 weeks gestation. The fetuses had crown rump lengths of 0.3, 1.7, 1.9, 2.5, 3.0, 4.0, 5.0, 8.0, 13.0, 15.0, 16.5, and 21.5 cm. Four of these fetuses were of undetermined sex, 3 were male and 5 were female. Tissue was obtained at autopsy from a female infant that expired after a 40 week gestation (6 lbs, 19 in.). The institutional clinical investigation committee reviewed and approved the study protocol in which these embryos and fetuses were obtained (UC protocol #4411).

### *Adult tissues*

Both frozen and formalin-fixed, paraffin-embedded tissues derived from surgical specimens were used for these studies. Normal adult tissues (followed by number of cases of each) analysed for HER-2/*neu* expression included lung ($n = 12$), salivary gland ($n = 4$), esophagus ($n = 6$), stomach ($n = 13$), small intestine ($n = 8$), large intestine ($n = 10$), liver ($n = 10$), gall bladder ($n = 2$), spleen ($n = 2$), lymph node ($n = 7$), thymus ($n = 1$), pancreas ($n = 4$), pituitary ($n = 2$), thyroid ($n = 7$), adrenal gland ($n = 7$), kidney ($n = 16$), bladder ($n = 10$), testis ($n = 8$), prostate ($n = 12$), epididymus ($n = 2$), ovary ($n = 3$), skin ($n = 10$), bone ($n = 4$), muscle ($n = 10$) and brain ($n = 8$). In order to characterize HER-2/*neu* expression during the female reproductive cycle, tissue from normal vagina specimens of 21 cases, endometrium of 28 cases, fallopian tube of 21 cases, cervix of 21 cases, and breast of 21 cases, equally distributed throughout the menstrual cycle, were selected. Some of these cases have been reported previously in studies of either estrogen receptor (Press & Greene, 1984; Press *et al.*, 1984; Press *et al.*, 1986) or progesterone receptor expression in the female reproductive tract during the menstrual cycle (Press, Udove & Greene, 1988; Press & Greene, 1988). The normal breast samples were obtained from women

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undergoing augmentation or reduction mammoplasties. Menstrual cycle dating was determined from historical information, from the date of the first menses following surgery and from serum steroid hormone levels in blood obtained immediately prior to surgery. Only one normal breast sample obtained from a woman who previously had a hysterectomy did not have the menstrual cycle date established.

*Immunohistochemical assay*

All normal tissues available for the study were analysed by immunohistochemistry. A rabbit polyclonal antiserum (#60), specific for HER-2/*neu* protein (Slamon *et al.*, 1989b), was used with the peroxidase antiperoxidase technique. The immunohistochemical technique involved the sequential application of the following antibodies: primary rabbit anti-HER-2/*neu* antiserum #60 (1:1000 dilution), a secondary or bridging goat antirabbit IgG (1:50 dilution, half hour) (Sternberger-Mayer, Inc.) and a tertiary rabbit peroxidase-antiperoxidase antibody (1:50, half hour) (Sternberger-Mayer, Inc.). Each antibody incubation was followed by rinsing the tissue sections in phosphate buffered saline three times (5 min each). The sites of immunoprecipitate formation were identified using light microscopy following treatment with a chromogen, 3-3′-diaminobenzidine. Tissues were grouped into one of the following expression categories depending on the amount of membrane immunostaining: negative (−), weak (wk), distinct (1+), moderate (2+), and strong/intense (3+). These categories are similar to those previously described for breast and ovarian cancers (Slamon *et al.*, 1989a, b). We do not report percentage of immunoreactive cells since relatively homogeneous immunostaining was observed in specific cell types throughout frozen sections of normal tissues (approximately 90% of the cells of a particular type showing similar immunostaining properties). Similar observations were made in frozen sections of breast and ovarian tumors (Slamon *et al.*, 1989a, b).

Negative control serial sections were prepared using preimmune serum (1:1000 dilution) instead of the immune serum in the peroxidase anti-peroxidase technique. The specificity of the #60 antiserum was confirmed with competition studies using purified HER-2/*neu* protein (Slamon *et al.*, 1989b).

Immunostaining of frozen tissue sections, performed in a blinded fashion, was compared with immunostaining of formalin-fixed, paraffin-embedded tissue sections. Paraffin-embedded tissue sections were rehydrated by warming at 55–60°C for one hour and then transferring to xylene and a graded series of alcohols followed by phosphate buffered saline. The peroxidase antiperoxidase technique was performed as above except the incubation times with primary antibody differed. Frozen section localization of HER-2/*neu* protein was not improved by incubation beyond one hour; however, in tests of optimum conditions for immunostaining, overnight incubation resulted in stronger membrane staining of cells in paraffin-embedded sections. Otherwise, the immunostaining protocols were identical.

*Northern hybridization*

Identification of the 4.5 kb messenger RNA encoding the HER-2/*neu* protein was done by Northern blot hybridization of total RNA extracted from selected normal adult and fetal tissues. RNA was isolated and the Northern hybridization performed as previously described (Thomas, 1980; Slamon *et al.*, 1989a, b). RNA was extracted from 28 cases of normal adult endometrium distributed throughout the menstrual cycle, 4 cases of gestational endometrium, 2 cases each of adult and fetal kidney, 2 cases each of adult and fetal lung, 2 cases each of adult and fetal liver, one case each of adult and fetal colon, three cases of fetal brain, one case of adult brain, one case of fetal heart, one case of fetal lower limb, and one case of adult spleen.

*Southern hybridization*

HER-2/*neu* gene copy level was determined by Southern hybridization of EcoRI digested DNA from selected normal adult and fetal tissues. The DNA was extracted from the same cases listed for Northern blot analyses. The Southern hybridization was performed as previously described (Southern, 1973; Slamon *et al.*, 1989a; Slamon & Clark, 1988). Autoradiograms were quantitated by soft laser densitometry. Autoradiograms obtained with probes for the myeloperoxidase and p53 genes, found on the long and short arms of chromosome 17, respectively, served as controls to demonstrate that similar amounts of DNA had been loaded into each lane.

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**References**

Bartow, S., Hildebrand, R., Boyd, M. & Press, M.F. (1989). *Lab. Invest.*, **62**, 8A, 1990. (abstract)

Cohen, J.A., Weiner, D.B., More, K.F., Kokai, Y., Williams, W.V., Maguire, H.C., Jr, LiVolsi, V.A. & Greene, M.I. (1989). *Oncogene*, **4**, 81–88.

Coussens, L., Yang-Feng, T.L., Liao, Y.-C., Chen, E., Gray, A., McGrath, J., Seeburg, P.H., Libermann, T.A., Schlessinger, J., Francke, U., Levinson, A. & Ullrich, A. (1985). *Science*, **230**, 1132–1139.

De Potter, C.R., Daele, S.V., Van DeVijver, M.J., Pauwels, C., Maertens, G., De Boever, J., Vandekerckhove, D. & Roels, H. (1989). *Histopathology*, **15**, 351–362.

DiFiore, P.P., Pierce, J.H., Kraus, M.H., Segatto, O., King, C.R. & Aaronson, S.A. (1987). *Science*, **237**, 178–182.

Drebin, J.A., Link, V.C., Stern, D.F., Weinberg, R.A. & Greene, M.I. (1985). *Cell*, **41**, 695–706.

Ekblom, P. (1989). *FASEB J.*, **3**, 2141–2150.

Gusterson, B.A., Gullick, W.J., Venter, D.J., Powles, T.J., Elliott, C., Ashley, S., Tidy, A., Harrison, S. (1988). *Mol. Cell. Probes*, **2**, 383–391.

Hudziak, R.M., Schlessinger, J. & Ullrich, A. (1987). *Proc. Natl. Acad. Sci. USA*, **84**, 7159–7163.

King, C.R., Kraus, M.H. & Aaronson, S.A. (1985). *Science*, **229**, 974–976.

Kokai, Y., Cohen, J.A., Drebin, J.A. & Greene, M.I. (1987). *Proc. Natl. Acad. Sci. USA*, **84**, 8498–8501.

Kraus, M.H., Popescu, N.C., Amsbaugh, S.C. & King, C.R. (1987). *EMBO J.*, **6**, 605–610.

Mori, S., Akiyama, T., Yamada, Y., Morishita, Y., Sugawara, I., Toyoshima, K. & Yamamoto, T. (1989). *Lab. Invest.*, **61**, 93–97.

Muller, W.J., Sinn, E., Pattengale, P.K., Wallace, R. & Leder, P. (1988). *Cell*, **54**, 105–115.

Paik, S., Hazan, R., Fisher, E.R., Sass, R.E., Fisher, B.,

PHIGENIX
Exhibit 1029-23

Redmond, C., Schlessinger, J., Lippman, M.E. & King, C.R. (1990). *J. Clin. Oncol.*, **8**, 103–112.

Press, M.F. & Greene, G.L. (1984). *Lab. Invest.*, **50**, 480–486.

Press, M.F., Nousek-Goebl, N.A., King, W.J., Herbst, A.L. & Greene, G.L. (1984). *Lab. Invest.*, **51**, 495–504.

Press, M.F., Nousek-Goebl, N.A., Bur, M. & Greene, G.L. (1986). *Am. J. Pathol.*, 123, 280–292.

Press, M.F. & Greene, G.L. (1988). *Endocrinology*, **122**, 1165–1175.

Press, M.F., Udove, J.A. & Greene, G.L. (1988). *Am. J. Pathol.*, **131**, 112–124.

Quirke, P., Pickles, A., Tuzi, N.L., Mohamdee, O. & Gullick, W.L. (1989). *Brit. J. Cancer*, **60**, 64–69.

Ro, J., El-Neggar, A., Ro, J.Y., Blick, M., Frye, D., Fraschini, G., Fritsche, H. & Hortobagyi, G. (1989). *Cancer Res.*, **49**, 6941–6944.

Schechter, A.L., Stern, D.F., Vaidyanathan, L., Decker, S.J., Drebin, J.A., Greene, M.I. & Weinberg, R.A. (1984). *Nature*, **312**, 513–516.

Semba, K., Kamata, N., Toyoshima, K. & Yamamoto, T. (1985). *Proc. Natl. Acad. Sci. USA*, **82**, 6497–6501.

Shih, C., Padhy, L.C., Murray, M. & Weinberg, R.A. (1981). *Nature*, **290**, 261–264.

Slamon, D.J. & Clark, G.M. (1988). *Science*, **240**, 1796–1798.

Slamon, D.J., Clark, G.M., Wong, S.G., Levin, W.J., Ullrich, A. & McGuire, W.L. (1987). *Science*, **235**, 177–182.

Slamon, D.J., Godolphin, W., Jones, L.A., Holt, J.A., Wong, S.G., Keith, D.E., Levin, W.J., Stuart, S.G., Udove, J., Ullrich, A. & Press, M.F. (1989a). *Science*, **244**, 707–712.

Slamon, D.J., Press, M.F., Godolphin, W., Ramos, L., Haran, P., Shek, L., Stuart, S.G. & Ullrich, A. (1989b). *Cancer Cells*, **7**, 371–380.

Southern, E.M. (1975). *J. Mol. Biol.*, **98**, 503–517.

Thomas, P.S. (1980). *Proc. Natl. Acad. Sci. USA*, **77**, 5201–5205.

Van de Vijver, M.J., Peterse, J.L., Mooi, W.J., Wisman, P., Lomans, J., Dalesio, O. & Nusse, R. (1988). *N. Engl. J. Med.*, **319**, 1239–1245.

Wright, C., Angus, B., Nicholson, S., Sainsbury, R.C., Cairns, J., Gullick, W.J., Kelly, P., Harris, A.L. & Horne, C.H.W. (1989). *Cancer Res.*, **49**, 2087–2090.

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# EXHIBIT C

[CANCER RESEARCH 39, 3152-3155, August 1979]
0008-5472/79/0039-0000$02.00

# Cell Cycle Phase-specific Cytotoxicity of the Antitumor Agent Maytansine[1]

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## ABSTRACT

The objective of this investigation was to study the effects of maytansine on the cell cycle kinetics of HeLa cells. The results of this study indicate that maytansine is a very potent mitotic inhibitor and that it has no effect on macromolecular synthesis. Maytansine-induced cytotoxicity was dependent upon the position of the cell in the cell cycle. Mitotic and $G_2$ cells are most sensitive to this agent, while $G_1$ phase cells are the most resistant, with S-phase cells being intermediate. Small ($0.82 \times 10^{-8}$ M) fractionated doses given at an interval of 8 hr have been found to be more cytotoxic than was a large ($1.64 \times 10^{-8}$ M) single dose. In evaluating the drug combinations, we observed that the schedule in which 1-β-D-arabinofuranosylcytosine treatment was followed by maytansine treatment exhibited greater cell kill than the reverse sequence. No schedule-dependent effects were observed when maytansine was tried in combination with Adriamycin.

## INTRODUCTION

Maytansine, a naturally occurring ansa macrolide, isolated from the East African shrub *Maytenus serrata* (4, 5), has been reported to have significant antitumor activity against several experimental animal tumors, including P388 lymphocytic leukemia, B16 melanoma, and Walker 256 carcinoma (11). The antitumor activity of maytansine appears primarily to be due to its stathmokinetic effects, as in the case of *Vinca* alkaloids (12). Phase 1 clinical trials with maytansine in our department at M. D. Anderson Hospital and Tumor Institute appear to be promising because the antitumor activity of maytansine in patients with melanoma, breast carcinoma, and head and neck clear cell carcinoma is associated with little or no myelosuppression (2). Responses were also observed by other investigators in patients with acute lymphocytic leukemia, non-Hodgkin's lymphoma, ovarian cancer (3), and carcinoma of the breast (1). Maytansine is now in Phase 2 clinical trials.

Since maytansine is associated with some dose-dependent gastrointestinal toxicity (2, 3), we decided to study the effects of scheduling, dose, and dose fractionation of maytansine alone and in combination with ara-C[3] or Adriamycin on HeLa cells *in vitro*.

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[2] To whom requests for reprints should be sent.

[3] The abbreviations used are: ara-C, 1-β-D-arabinofuranosylcytosine; dThd, thymidine.

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## MATERIALS AND METHODS

**Cells.** HeLa cells used in this study were grown in Lux plastic dishes as monolayer cultures in McCoy's Medium 5A supplemented with 16% heat-inactivated fetal calf serum and 1% penicillin (10,000 units/ml):streptomycin (10,000 μg/ml). These cells have a cell cycle time of approximately 22 hr and a plating efficiency of about 90%.

**Cell Synchrony.** HeLa cells were synchronized in S phase by the excess dThd (2.5 mM) double-block method (8). Cells in S and $G_2$ phases were obtained by trypsinizing monolayer cultures at 1 and 7 hr, respectively, after reversal of the second dThd block. A pulse label of 30 min with [$^3$H]dThd gave a labeling index of 95% for S-phase population and 15% for the $G_2$ population. The mitotic index in these populations was less than 1%. Mitotic HeLa cells of 98% purity or better were obtained by selective detachment after the exposure to nitrous oxide (at 80 psi and 37°) of a monolayer culture that was partially synchronized into S phase by a single dThd block (7). $G_1$ phase cells were obtained by incubating the $N_2O$-arrested mitotic cells under regular culture conditions for 3 hr. During this 3-hr incubation, the mitotic index decreased from 98% to below 5%, indicating the successful completion of mitosis following reversal of the $N_2O$ block.

**Drugs.** Maytansine (NSC 153858), ara-C (NSC 63878), and Adriamycin (NSC 123127) were supplied by the Drug Development Branch, National Cancer Institute, NIH. Stock solutions of these drugs were freshly prepared just before use and then serially diluted in complete culture medium to obtain the desired concentrations.

**Cell Cycle Kinetics.** A culture in exponential growth was trypsinized and plated in a number of Lux 35-mm plastic dishes at $2 \times 10^5$ cells/dish about 20 hr before the experiment. The experiment was begun by replacing the medium in the dishes with fresh medium containing maytansine. The drug concentrations studied were 0, 0.5, 1.0, 2.0, 4.1, 8.2, 16.4, and 32.8 nM. For each concentration, there were 2 dishes, one for an 18-hr continuous treatment and the other for a pulse treatment of 60 min (followed by a wash to remove the drug) and a posttreatment incubation of 17 hr in regular medium. At the end of this period, the cells were collected by trypsinization, deposited directly on clean slides by the use of a cytocentrifuge, fixed in absolute methanol:glacial acetic acid (3:1, v/v), stained with acetoorcein, and scored for the percentage of cells in mitosis. Five hundred cells were scored for each point. The mitotic accumulation was plotted as a function of dose. The data presented represent an average of 3 experiments.

**Dose-Survival Studies.** The procedures for drug treatment and the determination of plating efficiency have been described previously (9). HeLa cells in exponential growth, which were trypsinized and plated in a number of dishes the day before



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the experiment, were exposed to various concentrations of the drug for 1 hr or more, depending upon the purpose of the experiment. At the end of the treatment, medium containing the drug was removed, and cells were washed with drug-free medium, trypsinized, plated for colonies, and incubated for 10 days. The number of colonies observed in the treatments was expressed as a percentage of the value for the untreated control. The plating efficiency of the controls was 85 ± 7% (S.D.).

**Evaluation of Drug Combinations.** For the *in vitro* evaluation of combined drug effects, a random population of HeLa cells was exposed first to one drug that was then removed by washing before the second drug was added to the medium. Soon after the drug treatments, cells were washed with regular medium, trypsinized, and plated for colonies. In this study, 2-drug combinations, maytansine:ara-C and maytansine:Adriamycin, were examined. The various schedules included maytansine followed by either ara-C or Adriamycin and the reverse sequence. The duration of exposure of cells to maytansine and Adriamycin was 60 min. However, with ara-C, the treatment was 16 hr because it has been shown that a 16-hr incubation of a random population of HeLa cells with a sublethal dose (0.8 μg/ml) of ara-C reversibly blocked about 90% of the cells in S phase (9).

## RESULTS

**Effect on Cell Cycle Traverse and Macromolecular Synthesis.** The purpose of this set of experiments was to determine the optimum dose and duration of treatment of HeLa cells with maytansine to produce the maximum cytotoxic effects. The primary effect of maytansine on HeLa cells was the arrest of cells in metaphase. The degree of mitotic accumulation in a random population of HeLa cells after 18 hr of continuous exposure or after 17 hr of incubation following a 1-hr treatment with maytansine is presented in Chart 1. In general, the effects of maytansine on mitotic accumulation were similar to those of Colcemid. The effects of a pulse (60 min) exposure of cells to the drug were reversible at lower (0.05 to 0.2 × $10^{-8}$ M) but not at higher concentrations.

Maytansine has no inhibitory effect on the rate of incorporation of tritium-labeled precursors into DNA, RNA, and protein during a 3-hr period (Table 1). However, there was some increase in the incorporation of [$^3$H]leucine in the presence of Colcemid or maytansine in the medium.

**Effect on Cell Survival.** The effect of a 1-hr treatment with various concentrations of maytansine on the plating (cloning) efficiency of HeLa cells was studied. Initially, the plating efficiency decreased with an increase in dose, but it soon reached a plateau (Chart 2). Further increase in dose had little or no effect on survival until the concentration reached 6.56 × $10^{-8}$ M. However, an increase in the duration of treatment resulted in a decrease in the plating efficiency (Chart 3).

**Effect of Dose Fractionation on Survival.** Based on the dose-survival curve in Chart 2, a maytansine concentration of 1.64 × $10^{-8}$ M was selected. This dose was fractionated into 2 doses (0.82 × $10^{-8}$ M each), and each was applied for 1 hr, with or without an interval between them. These results indicate that the longer the interval between the split doses (up to a maximum of 8 hr studied) the greater was the decrease in cell survival (Chart 4).

**Cell Cycle Phase-Specific Effects of Maytansine on Plating Efficiency.** When HeLa cells synchronized in various phases of the cell cycle were exposed to maytansine (1.64 × $10^{-8}$ M) for 1 hr, the percentage of survival varied depending upon the phase of the synchronized population (Chart 5). The greatest drug sensitivity was observed in mitotic populations followed by $G_2$, S, and $G_1$, in order of decreasing sensitivity.

***In Vitro* Evaluation of Drug Combinations.** In view of the cell

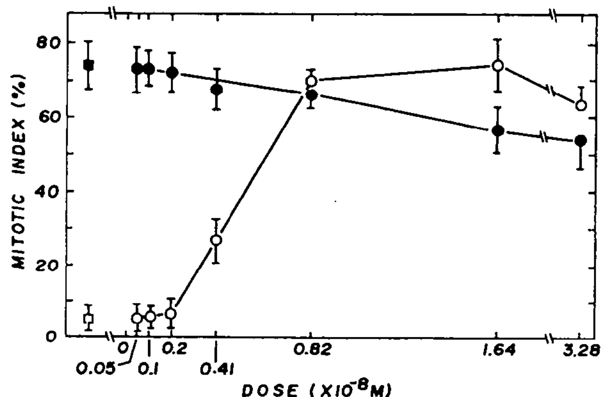


Chart 1. Effect of various concentrations of maytansine on the mitotic accumulation of HeLa cells in exponential growth. ○, a pulse treatment; cells were exposed to maytansine for 60 min; the drug was removed by washing and then incubated in regular medium for 17 hr. ●, cells were incubated with maytansine for 18 hr. Cells exposed to Colcemid (1.37 × $10^{-7}$ M or 0.05 μg/ml) served as a control to monitor the antimitotic effects of maytansine. ■, 18 hr continuous Colcemid treatment; □, 1 hr Colcemid treatment followed by 17 hr incubation in regular medium. *Bars*, S.D.

Table 1

*Effect of maytansine on the incorporation of tritium-labeled precursors in DNA, RNA, and protein*

The data represent the average of 4 experiments.

| Treatment | Relative cpm/$10^6$ cells | | |
|---|---|---|---|
| | [$^3$H]dThd | [$^3$H]Uridine | [$^3$H]Leucine |
| Control | 100 | 100 | 100 |
| Colcemid (0.05 μg/ml) | 106.4 ± 4.39[a] | 94.5 ± 7.20 | 121.2 ± 11.10 |
| Maytansine (0.656 × $10^{-9}$ M; 0.5 ng/ml) | 102.7 ± 8.10 | 111.3 ± 10.60 | 152.0 ± 13.8 |

[a] Mean ± S.D.

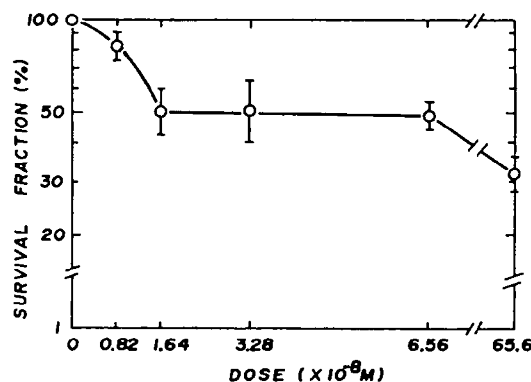


Chart 2. Effect of a 1-hr treatment with various concentrations of maytansine (○) on the plating efficiency of HeLa cells in exponential growth. *Bars*, S.D.



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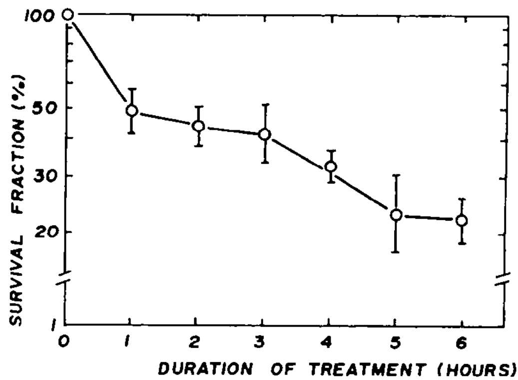


Chart 3. Effect of the duration of maytansine ($1.64 \times 10^{-8}$ M) (O) treatment on the plating efficiency of HeLa cells in exponential growth. *Bars*, S.D.

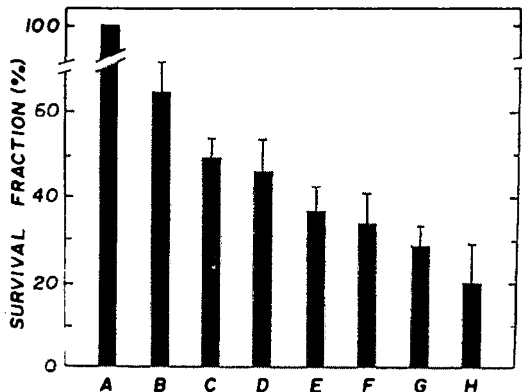


Chart 4. Effect of dose fractionation and the duration of interval between the treatments on the survival of HeLa cells in exponential growth. *A*, untreated control; *B*, maytansine treatment ($0.82 \times 10^{-8}$ M) for 1 hr; *C*, maytansine treatment ($1.64 \times 10^{-8}$ M) for 1 hr. For dose fractionation studies, 2 treatments of maytansine at $0.82 \times 10^{-8}$ M for 60 min each were applied at zero (*D*), 2- (*E*), 4- (*F*), 6- (*G*), and 8- (*H*) hr intervals. *Bars*, S.D.

cycle phase-specific cytotoxicity of maytansine, we decided to study whether there would be any increase in cell kill by synchronizing with a low dose of ara-C and then exposing the cells to maytansine. Schedule-dependent cytotoxic effects were observed in the combination of maytansine with ara-C but not with Adriamycin (Chart 6). Exposure of cells to ara-C for 16 hr followed by a 1-hr treatment with maytansine reduced the plating efficiency to about 25%, as compared with 41% survival when the sequence was reversed. The data presented are the averages of 3 experiments.

## DISCUSSION

The results of this study indicate that maytansine is primarily a mitotic inhibitor. As a mitotic inhibitor, it is effective over a wide range of concentrations (Chart 1). The lowest effective concentration (0.5 nM) for maytansine in HeLa cells is about 200 times smaller than that for Colcemid ($1.37 \times 10^{-7}$ M). Similarly, maytansine has been shown to be at least 100 times more potent as an antimitotic agent than vincristine in sea urchin eggs (10).

Continuous treatment of HeLa cells with higher doses of maytansine may slow down the progression of cells through the cell cycle to some extent, as indicated by a slight reduction in the degree of mitotic accumulation (Chart 1). Such retarding effects are not observed if the duration of treatment is limited to 1 hr. A 60-min treatment with concentrations of maytansine above $0.82 \times 10^{-8}$ M produces a mitotic block in HeLa cells that remained irreversible up to 18 hr. At concentrations of $0.2 \times 10^{-8}$ M or lower, the antimitotic effects are quickly reversible by washing and resuspending the cells in drug-free medium. Similar results were obtained with murine leukemia cells by Wolpert-Defilippes *et al.* (12).

A 3-hr incubation of a random culture of HeLa cells with maytansine at concentrations of $6.6 \times 10^{-8}$ M had no effect on the incorporation of [$^3$H]dThd and [$^3$H]uridine into DNA and RNA, respectively. A measurable increase in the uptake of [$^3$H]leucine into both Colcemid- and maytansine-treated cells is unexpected. Probably, these agents, due to their disorganizing effects on the cytoskeleton, may increase the permeability of

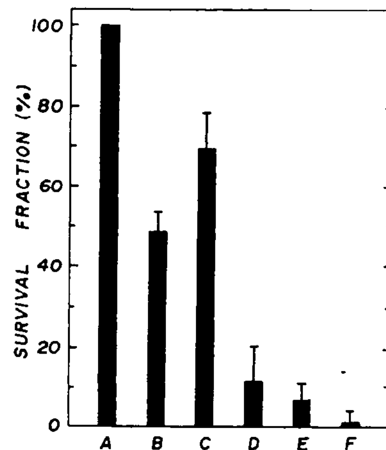


Chart 5. Effect of maytansine ($1.64 \times 10^{-8}$ M for 60 min) on the plating efficiency of HeLa cells synchronized in various phases of the cell cycle. The number of colonies observed in the treatment is expressed as a percentage of the untreated control for each phase of the cell cycle. *A*, control, a random culture. Maytansine treatments: *B*, random culture; *C*, $G_1$ phase; *D*, S phase; *E*, $G_2$ phase; *F*, mitotic populations. *Bars*, S.D.

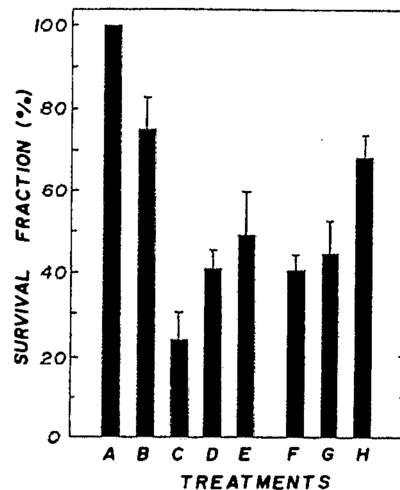


Chart 6. Effect of maytansine in combination with ara-C or Adriamycin on the survival of HeLa cells in exponential growth. The treatments included ara-C (0.8 µM) for 16 hr, Adriamycin (0.2 µg/ml) for 1 hr, and maytansine ($1.64 \times 10^{-8}$ M) for 1 hr. *A*, untreated control; *B*, ara-C treatment; *C*, ara-C followed by maytansine; *D*, maytansine followed by ara-C; *E*, maytansine alone; *F*, maytansine followed by Adriamycin; *G*, Adriamycin followed by maytansine; *H*, Adriamycin alone. *Bars*, S.D.



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the cell membranes to [$^3$H]leucine, thus facilitating greater uptake and consequently greater incorporation of label into the protein than occurs in the control. These results are at variance with those of Wolpert-Defilippes *et al.* (12), who reported that, in murine leukemic cells, DNA synthesis was inhibited to the greatest extent. However, they measured the uptake of labeled precursors into DNA, RNA, and protein by pulse-labeling of cells that were incubated with maytansine over a period of 12 hr. A 12-hr exposure to maytansine at $10^{-7}$ M would certainly block more than 50% of these cells in mitosis. The arrest of a significant fraction of the cell population in mitosis would result in a considerable reduction in the incorporation of label into DNA, RNA, and protein when compared with untreated control cultures.

The dose-survival curve for maytansine (Chart 2) quickly reaches a plateau with an increase in the concentration, indicating that there are 2 cell types in the population, one sensitive and the other relatively resistant. However, with a fixed dose, there is a direct correlation between the duration of treatment and the percentage increase in cell kill (Chart 3). Dose fractionation studies reveal that small fractionated doses are more cytotoxic than is a large single dose (Chart 4). The longer the interval between the fractionated doses, the lower is the plating efficiency up to 8 hr. These results suggest that most of the sensitive fraction in the cell population is killed by the first treatment, whereas the second treatment kills those that move from a resistant to a more sensitive phase of the cell cycle.

Studies with synchronized populations reveal that cells in mitosis are the most sensitive and those in $G_1$ are the most resistant to this agent (Chart 5). The closer the cell is to mitosis, the more sensitive it is to the cytotoxic effects of maytansine. Depolymerization and inhibition of polymerization of tubulin has been shown to account for the antimitotic effects of maytansine (10). Since oxidation of the sulfhydryl groups in tubulin inhibits its polymerization, the effect of maytansine may be due to its binding to these groups (10). It is also conceivable that tubulin, which is the most important constituent of the mitotic apparatus, accumulates gradually during the cell cycle reaching a peak at the beginning of mitosis (6). Thus, cells in $G_2$ and mitosis would have a full complement of these proteins, whereas those in $G_1$ would have the least proteins, with S-phase cells being intermediate. As soon as the cells were exposed to cytotoxic doses of maytansine, the spindle protein (tubulin) would be inactivated (depolymerized) by the irreversible binding of the drug. If the drug were removed after a brief exposure of 60 min, cells in $G_1$ and to some extent those in S could synthesize new tubulin and thus overcome the antimitotic effects of maytansine. Hence, $G_1$ cells would be more resistant to maytansine than those in other phases of the cell cycle, as we have observed. In the light of these observations, we can explain the pattern of the dose-survival curve (Chart 1) as follows. The initial sharp decrease in the plating efficiency represents the killing of cells that were in the sensitive phases of the cell cycle by low concentrations of maytansine. On the other hand, the plateau is represented by the more resistant $G_1$ fraction, which constitutes about 50% of the population and remains unaffected over a relatively wide range of drug concentrations.

*In vitro* evaluation of the cytotoxic effects of maytansine in combination with ara-C revealed schedule-dependent effects (Chart 6). Administration of maytansine following a 16-hr ara-C treatment is more cytotoxic than the reverse sequence. This could be due to the synchronization of cells in S phase by the ara-C treatment. Our results (Chart 5) indicate that S-phase cells are more sensitive than those in $G_1$. Since Adriamycin does not induce cell synchrony, the sequence of administration of Adriamycin and maytansine made no difference in cell survival.

## ACKNOWLEDGMENTS

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## REFERENCES

1. Blum, R. H., and Kahert, T. Maytansine. A Phase I study of an ansa macrolide with antitumor activity. Cancer Treat. Rep., *62:* 435–438, 1978.
2. Cabanillas, F., Rodriguez, V., Hall, S. W., Burgess, M. A., Bodey, G. P., and Freireich, E. J. Phase I study of maytansine using a 3-day schedule. Cancer Treat. Rep., *62:* 425–428, 1978.
3. Chabner, B. A., Levine, A. S., Johnson, B. L., and Young, R. C. Initial clinical trials of maytansine, an antitumor plant alkaloid. Cancer Treat. Rep., *62:* 429–433, 1978.
4. Kupchan, S. M., Komoda, Y., Brantman, A. R., Dailey, R. G., Jr., and Zimmerly, V. A. Novel maytansinoids. Structural interrelations and requirements for antileukemic activity. J. Am. Chem. Soc., *96:* 3706–3708, 1974.
5. Kupchan, S. M., Komoda, Y., Court, W. A., Thomas, G. J., Smith, R. M., Karim, A., Gilmore, C. G., Haltiwanger, R. C., and Bryan, R. F. Maytansine, a novel antileukemic ansa macrolide from *Maytenus ovatus*. J. Am. Chem. Soc., *94:* 1354–1356, 1972.
6. Lawrence, J. H., and Wheatley, D. N. Synthesis of microtubule protein in HeLa cells approaching division. Cytobios, *13:* 167–179, 1975.
7. Rao, R. N. Mitotic synchrony in mammalian cells treated with nitrous oxide at high pressure. Science, *160:* 774–776, 1968.
8. Rao, P. N., and Engelberg, J. Effects of temperature on the mitotic cycle of normal and synchronized mammalian cells. *In:* I. L. Cameron and G. M. Padilla (eds.), Cell Synchrony—Biosynthetic Regulation, pp. 332–352. New York: Academic Press, Inc., 1966.
9. Rao, P. N., Freireich, E. J., Bodey, G. P., Gottlieb, J. A., and Smith, M. L. *In vitro* evaluation of 1-$\beta$-D-arabinofuranosylcytosine-$\beta$-2'-deoxyguanosine combination chemotherapy. Cancer Res., *34:* 2539–2543, 1974.
10. Remillard, S., and Rebhun, L. I. Antimitotic activity of the potent tumor inhibitor maytansine. Science, *189:* 1002–1005, 1975.
11. Sieber, S. M., Wolpert, M. K., Adamson, R. H., Cysyk, R. L., Bono, V. H., and Johns, D. G. Experimental studies with maytansine—a new antitumor agent. Bibl. Haematol., *43:* 495–500, 1976.
12. Wolpert-Defilippes, M. K., Adamson, R. H., Cysyk, R. L., and Johns, D. G. Initial studies on the cytotoxic action of maytansine, a novel ansa macrolide. Biochem. Pharmacol., *24:* 751–754, 1975.



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# EXHIBIT D

target (Fig. 2B) or 180° out of phase with it (Fig. 2D). In both situations the unit continued to modulate in relation to the track-target's velocity, increasing its rate with the leftward movement. All 13 track-related Purkinje cells tested on all of these paradigms showed essentially the same pattern of firing. In the few units examined under conditions in which the movements of the target and chair were completely dissociated, track-target motion relative to earth-fixed surroundings was still the crucial factor. Irregularities in firing which are apparent as noise in the reciprocal interval plots tend to obscure the correlation with target velocity; they may reflect microvariations in the monkey's performance which are beyond the resolution of our oculogram recordings but are nonetheless significant.

That these neural correlates of visual tracking in the primate flocculus are probably not mere epiphenomena but may instead reflect some vital role in the programming of pursuit eye movements is suggested by the deficits which follow lesions in this area. Severe, and relatively specific, impairments of smooth pursuit eye movements are often associated with cerebellar atrophy in man (*8*), and total cerebellectomy in the mature monkey results in the permanent loss of such eye movements (*9*). Deficits in optokinetic responses after bilateral flocculectomy in monkeys have been reported, but, unfortunately, the animals were not examined specifically for pursuit eye movements of the kind under consideration here (*10*).

We hypothesize that Purkinje cell output from the primate flocculus provides oculomotor centers with target velocity information essential for visual tracking and represents an output of the smooth pursuit subsystem. In addition, we have some data which may help explain how the flocculus generates these velocity command signals. Most units in the flocculus showed no evidence of a CS, and we assume that they represent one or another of the various input elements known to influence Purkinje cells. The majority of such units fired vigorously in relation to saccadic eye movements often with both transient and tonic components but no apparent special concern with tracking; others seemed to be driven by vestibular inputs, modulating nicely in phase with chair (that is, head) velocity. We became aware of a class of visually driven units lacking CS's; these were especially sensitive to retinal image slip in the region of the fovea and often were more responsive to ipsilateral target movements. Firing of these units may be the putative error signal that ultimately sustains pursuit eye movements. These units closely resemble those recently described for the monkey's nucleus of the transpeduncular tract (*11*), a part of the accessory optic system; at least in the rabbit, this tract projects to the flocculus (*12*). Signal processing in the pursuit system may require a precise velocity representation of the target (*13*); we propose that this is the function of the Purkinje cells in the primate flocculus. A true neuronal facsimile of the track target's absolute velocity would require the summing of three signals: velocity of the target's retinal image (target motion relative to eye motion), eye velocity (eye motion relative to head motion), and head velocity (head motion relative to earth motion); we know that information concerning the first and last of these reaches the flocculus, and the second might easily be derived from the numerous inputs related to eye movements. A possible complication arises if the system has predictive capabilities, since the tracking waveforms contrived in our study were usually highly periodic (sinusoids and linear ramps).

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**References and Notes**

1. C. Rashbass, *J. Physiol.* **159**, 326 (1961).
2. N. Sugie and M. Wakakuwa, *IEEE (Inst. Elec. Electron. Eng.) Trans. Syst. Sci. Cyb.* **6**, 103 (1970). This study on man showed that eye-tracking performance is independent of head movement. No similar data are available for the monkey, although J. Dichgans *et al.* [*Brain Res.* **71**, 225 (1974)] attest to the efficacy of the vestibulo-ocular reflex in this species.
3. R. H. Wurtz, *J. Neurophysiol.* **32**, 975 (1969).
4. E. V. Evarts, *Electroencephalogr. Clin. Neurophysiol.* **24**, 83 (1968).
5. M. Ferin, R. A. Grigorian, P. Strata, *Exp. Brain Res.* **12**, 1 (1971).
6. About one-quarter of the units identified as Purkinje cells showed no consistent modulation with any of the parameters considered in our study, except for rather weak, transient pauses during saccadic movements. Some of these neurons may have been involved in vertical tracking, a possibility we did not investigate.
7. That Purkinje cell activity in primate flocculus modulates when the oscillating animal tracks a target moving with him but not when the target is stationary was first reported by S. G. Lisberger, and A. F. Fuchs [*Brain Res.* **69**, 347 (1974)].
8. J. Corvera, G. Torres-Courtney, G. Lopez-Rios, *Ann. Otol. Rhinol. Laryngol.* **82**, 855 (1973); R. W. Baloh, H. R. Konrad, V. Honrubia, *Neurology* **25**, 160 (1975); D. S. Zee *et al.*, *Trans. Am. Neurol. Assoc.*, in press.
9. G. Westheimer and S. M. Blair, *Invest. Ophthalmol.* **12**, 618 (1973).
10. S. Takemori and B. Cohen, *Brain Res.* **72**, 213 (1974).
11. G. Westheimer and S. M. Blair, *Invest. Ophthalmol.* **13**, 533 (1974).
12. K. Maekawa and J. I. Simpson, *J. Neurophysiol.* **36**, 649 (1973). The visual responses in the rabbit flocculus are mediated by climbing fibers, in contrast to the presumed mossy fiber responses reported here.
13. L. R. Young, in *The Control of Eye Movements*, P. Bach-y-Rita and C. C. Collins, Eds. (Academic Press, New York, 1971), p. 429.

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## Antimitotic Activity of the Potent Tumor Inhibitor Maytansine

Abstract. *Maytansine, at* $6 \times 10^{-8}$M, *irreversibly inhibits cell division in eggs of sea urchins and clams. It causes the disappearance of a mitotic apparatus or prevents one from forming if added at early stages. Maytansine does not affect formation of the mitotic organizing center but does inhibit in vitro polymerization of tubulin. Maytansine and vincristine inhibit in vitro polymerization of tubulin at about the same concentrations, but vincristine is about 100 times less effective as an inhibitor of cleavage in marine eggs.*

Maytansine, a novel ansa macrolide (Fig. 1), isolated from various *Maytenus* species, is an antitumor agent (*1*) that significantly inhibits mouse P-388 lymphocytic leukemia in dosages of micrograms per kilogram of body weight, and is active over a 50- to 100-fold dosage range. Maytansine also shows significant inhibitory activity against the L-1210 mouse leukemia, the Lewis lung carcinoma, and the B-16 melanocarcinoma murine tumor systems. This agent is being tested toxicologically in preparation for clinical trials (*2*).

We report now on the antimitotic effects of maytansine. At a concentration of $6 \times 10^{-8}M$, it totally inhibited cleavage in sea urchin eggs when applied at fertilization (*3*). At $4 \times 10^{-8}M$, 10 to 20 percent of the eggs divided (although cleavage was somewhat irregular). The remaining eggs formed irregular furrows that either did not separate equal-sized blastomeres or they subsequently retracted. When the eggs were treated with $10^{-8}M$ (or less) maytansine, the cleavage time, cleavage pattern, and later development were normal.

The critical time in egg development for inhibition of cleavage by $10^{-7}M$ maytansine was determined by adding the drug at 5-minute intervals, from the time of fertilization to first cleavage. Cleavage was totally inhibited when the drug was added at any time during the first half of the cleavage period; after that, an increasing number of cells went through some form of cleavage. However, even when the drug was added 10 minutes prior to cytokinesis, approximately 40 percent of the eggs did



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not cleave, and those that did, looked irregular and did not cleave a second time. When drug was added to unfertilized eggs for at least 1 hour, it could be removed prior to fertilization, with normal cleavage and development following. Drug added immediately after fertilization could be removed by repeated washings in seawater up to 20 minutes after fertilization, with minimal effects on cleavage pattern or rate. If removed between 20 and 30 minutes, irregular cleavages occurred; and if removed 30 minutes or more after fertilization, cleavage (which occurs 60 to 90 minutes after this point in controls) was permanently inhibited. Both the minimum concentration and critical time for total inhibition of cleavage were the same for the eggs of the sea urchins *Strongylocentrotus purpuratus* and *Lytechinus pictus*.

We investigated the possibility that the critical process affected by maytansine is DNA synthesis by determining the incorporation of [$^3$H]thymidine (*4*). The rate and amount of DNA synthesis was the same in eggs treated continuously with $10^{-7}M$ maytansine from 10 minutes before fertilization as that in controls, even though cleavage was totally inhibited in treated eggs.

In sea urchin eggs treated with $10^{-7}M$ maytansine before fertilization or starting 10 to 15 minutes after fertilization, fusion of the male and female pronuclei never occurred. A sperm aster (SA) (*5*) did not form in the egg. Since this body is necessary for the transport of the pronuclei to the egg center (*5*), pronuclear fusion does not occur in its absence. Further, since microtubules form an integral part of the SA (*6*), these observations suggested that maytansine may interfere with microtubule formation or tubulin mobilization into the aster.

To investigate the possibility that the drug might prevent the formation of mitotic apparatuses (MA's) as well as SA's, we used clam eggs (from *Spisula solidissima*) because of the ease with which the MA can be seen with a polarization microscope and the rapidity of MA formation [the MA starts to form about 10 minutes after fertilization or activation (*7*)]. Eggs treated with $10^{-7}M$ maytansine in seawater were parthenogenetically activated with KCl-seawater solutions (*7*). Activation of the eggs was normal and rupture of the nuclear membrane occurred on schedule with drug treatment, but an MA did not form. When maytansine was applied as late as 10 minutes after activation, an MA was not obtained; but eggs treated with $10^{-8}M$ maytansine had normal MA's (similar to those of the controls). Examination of eggs activated in the presence of maytansine at concentrations between $10^{-8}M$ and $10^{-7}M$ revealed the following. At $3 \times 10^{-8}M$, the MA was about one-half normal length and width (Fig. 2, a and b); at $6 \times 10^{-8}M$ (a dose that completely blocked polar body formation) the MA was approximately one-third normal length and one-fourth normal width (Fig. 2c); at $10^{-7}M$ a very small birefringent MA was present in the center of the egg and could not be seen without compressing the egg (Fig. 2d). Thus, maytansine regulates the size of the MA in a dose-dependent manner.

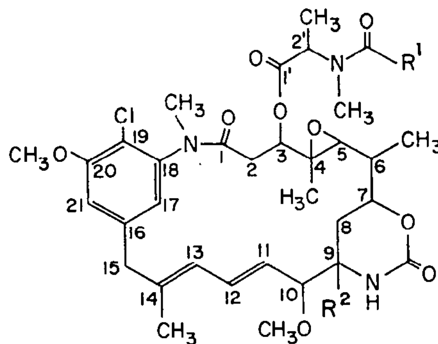


Fig. 1. Maytansine, $R^1 = CH_3$ and $R^2 = OH$; maytanbutine, $R^1 = CH(CH_3)_2$ and $R^2 = OH$; maytanbutine 9-*n*-propyl thioether, $R^1 = CH(CH_3)_2$ and $R^2 = SCH_2CH_2CH_3$.

We studied the question of whether maytansine could affect SA's in sea urchin or MA's in clam eggs once the structure had already formed. We found that SA's in sea urchin eggs and already formed MA's in clam eggs could be made to shrink in a dose-dependent manner by application of maytansine. Although exact measurements have not yet been made, the final size of the MA appears to be the same whether the MA formed in the presence of certain concentrations of maytansine or whether that concentration is added subsequent to MA formation.

Maytansine inhibits the formation of

Fig. 2. (a) Normal eggs of the surf clam *Spisula solidissima* observed with a polarization microscope. Contrast in the MA depends on its orientation relative to the axes of the microscope and can be reversed by rotation of the MA axis by 90° or change in angle of a compensator (×300). (b) *Spisula* egg treated with $3 \times 10^{-8}M$ maytansine prior to activation. The MA is reduced in size; the egg will form a first polar body, but it is considerably delayed in time (×300). (c) *Spisula* egg treated with $6 \times 10^{-8}M$ maytansine prior to activation. The first polar body never forms (×300). (d) *Spisula* egg treated with $2 \times 10^{-7}M$ maytansine prior to activation and compressed to visualize very small MA still remaining in the cytoplasm (×300). (e) *Spisula* egg treated with $6 \times 10^{-8}M$ maytansine plus 3 percent HG; compare with (c). The degree of augmentation with glycols decreases with increasing concentration of maytansine (×300). (f) *Spisula* egg in $2 \times 10^{-7}M$ maytansine treated with 3 percent HG to "develop" the MA after 30 minutes. Double MA indicates replication of the MTOC (×300). (g) Eggs of sea urchin *S. purpuratus* treated with $2 \times 10^{-7}M$ maytansine to eliminate SA (×190). (h) Augmented SA in *S. purpuratus* eggs treated with 5 percent HG (×190). (i) *S. purpuratus* eggs treated with 5 percent HG to which $4 \times 10^{-7}M$ maytansine is then added. SA is reduced in size in a dose-dependent manner similar to the phenomenon in MA of clam eggs (×190).



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the MA, either by inhibition of a mitotic organizing center (MTOC) (*8*), by interference with tubulin polymerization onto the center, or by some other mechanism. We tested the possibility that it might act on the MTOC by utilizing the long-chain glycol hexylene glycol (HG) since HG and other similar glycols cause augmentation of size and birefringence of SA's and MA's in a manner requiring the presence of an active MTOC (*9*). For these experiments both clam and sea urchin eggs were treated with maytansine before, at the same time as, or after treatment with 3 percent HG in seawater. At concentrations of $10^{-7}M$ maytansine an SA or MA was formed which was intermediate in size between normal and augmented SA's (Fig. 2, g to i) and MA's; at $3 \times 10^{-7}M$ maytansine, even the augmented structures could be caused to disappear. Further, clam eggs activated in maytansine and left for a half hour or so show multiple MA's or asters when augmented with glycols (Fig. 2f), suggesting that replication of the MTOC is not inactivated by the drug. Thus, maytansine does not appear to affect the ability of the MTOC to divide or to act as an organizing center for tubulin assembly. In this respect it acts in a manner similar to that of colchicine (*8*).

We then studied the effects of maytansine on brain tubulin polymerization (*10*), which can be used as a model for MA tubulin (*11*). Rabbit or pig brain tubulin was prepared by a slight modification of the method of Weisenberg (*10*) and purified through one or two cycles of polymerization. In each case polymerized microtubules were washed in buffer by centrifugation, a procedure that yields relatively pure tubulin after one cycle of polymerization, as judged by sodium dodecyl sulfate gel electrophoresis. Microtubules were depolymerized by cooling to 0°C in buffer in which guanosine triphosphate (GTP) was absent, and the resulting tubulin was used in subsequent experiments. Polymerization of tubulin was followed by turbidity increase at 310 nm (*12*) in a Gilford automatic recording spectrophotometer at 37°C. A sample of tubulin containing 1 m$M$ $Ca^{2+}$ and no added GTP was used as a blank, and turbidity was recorded as the difference between sample and blank. Polymerization was initiated by adding GTP to tubulin at 37°C. In initial experiments, maytansine was added to cold tubulin. Total inhibition of polymerization was found at $10^{-5}M$ maytansine in tubulin solutions of 4 to 5 mg/ml. With $5 \times 10^{-6}M$ maytansine the extent of polymerization of tubulin at 5 mg/ml was about 50 percent, as judged by turbidity at plateau levels. Maytansine added to polymerized tubulin caused a rapid decrease in turbidity to levels approximately the same as those attained when the same concentration was added before polymerization was started (Fig. 3). Inhibition of polymerization is about one-half at a mole ratio of 1 part of maytansine to 10 parts of tubulin, and total inhibition occurs at a mole ratio of 1 to 5.

To ascertain whether the mole ratio of maytansine to tubulin required for inhibition of in vitro polymerization could account for its antimitotic effects we assumed that maytansine would equilibrate between seawater and egg cytoplasm. The number of tubulin subunits in an MA the size of that of *S. purpuratus* is about $2 \times 10^8$ (*13*). Since the egg diameter is 75 $\mu$m, there should be about $1.2 \times 10^7$ molecules of maytansine in each egg at $10^{-7}M$ in the seawater if the drug equilibrates, and more if accumulation occurs. This is a ratio of 1 maytansine molecule to 16 tubulin molecules. Since brain tubulin has been shown to be a good model for MA tubulin (*11*), these results suggest that the inhibition of tubulin polymerization by maytansine can account for its in vivo antimitotic effects, given the nature of the calculations. Initial uptake experiments with [$^3$H]maytansine suggest a rapid accumulation of maytansine by eggs although the kinetics are not simple. They nevertheless suggest that enough maytansine enters the egg to support the above analysis.

Because the report of Wolper-Defilippes *et al.* (*14*) (which appeared as our experiments were nearing completion) suggested a relation of maytansine to vincristine in its antimitotic effects, we compared the two drugs for inhibition of tubulin polymerization in vitro and for inhibition of cleavage in vivo. Sea urchin eggs did not cleave in $10^{-5}M$ vincristine but formed irregular shallow furrows. At $5 \times 10^{-6}M$ vincristine cleavage was approximately normal; thus, maytansine is at least 100 times more potent as an antimitotic agent than vincristine. A difference in potency of about 15-fold was obtained with Chinese hamster ovary cells in culture. The molar concentration of vincristine necessary to inhibit polymerization of a given amount of brain tubulin, however, is actually somewhat lower for vincristine, and the kinetics of inhibition are very different. Colchicine inhibits tubulin polymerization in vitro at molar ratios even lower than that of maytansine (*15*) but requires a minimum dose of $10^{-4}M$ or more for inhibition of cleavage in sea urchin eggs (*16*). Why such marked differences of potency for MA inhibition in vivo exist in the face of the ability of all agents to inhibit brain tubulin polymerization in vitro to approximately the same degree is not known, but may be due to differences in uptake. However, another possibility suggests itself since we have found that oxidation of the sulfhydryl groups in tubulin inhibits its polymerization (*17*); conceivably maytansine acts by binding certain key sulfhydryls of tubulin.

The latter suggestion is supported by the readiness with which maytanbutine, a homologous maytansine ester, alkylated *n*-propane thiol to form the 9-*n*-propyl thioether (*18*). Presumably, maytansine may alkylate protein thiols in an analogous manner (*2*). We have also found that geld-

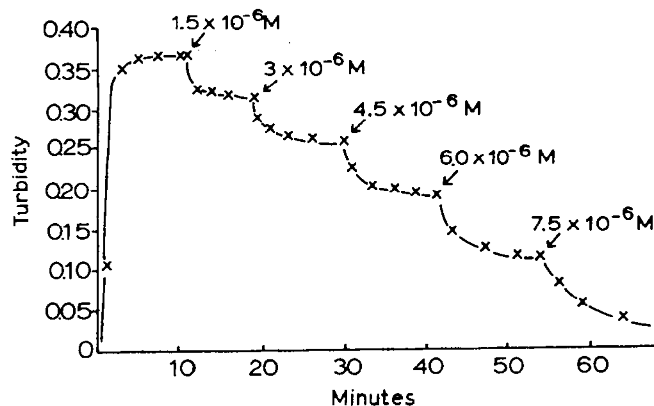


Fig. 3. Change in turbidity at 310 nm (ordinate) as a function of time. Brain tubulin was prepared as described in the text and was resuspended at 4.7 mg/ml ($4.27 \times 10^{-5}M$, assuming a molecular weight of 110,000 for tubulin). Polymerization was followed at 37°C by recording turbidity increase at 310 nm after addition of GTP (*12*) with the use, as a blank, of a sample of the same tubulin that was made 1 m$M$ in $CaCl_2$ and which had no added GTP (thus, polymerization was inhibited). Maytansine was added to sample and blank at the indicated points to give the final concentrations shown. The plateau levels reached were the same as those attained (within about 10 percent) if maytansine was added prior to initiation of polymerization by GTP. The turbidity in $7.5 \times 10^{-6}M$ maytansine eventually reached the baseline. In each case a portion of the sample was checked for birefringence with a polarizing strain detector. Birefringence only disappeared in the $7.5 \times 10^{-6}M$ sample when turbidity (compared to blank) vanished. We have found that birefringence of tubulin solutions always correlates with the presence of microtubules when viewed with the electron microscope. The ratio of tubulin to maytansine for decrease of turbidity by one-half was approximately 10 : 1 in four separate experiments performed at different tubulin concentrations. The ratio for complete depolymerization was approximately 5 : 1.



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anamycin, an ansa macrolide closely related to maytansine (*19*) but lacking the carbinolamide functionality involved in –SH alkylation (and which does not show antileukemic activity), is 1000 times less effective in inhibition of sea urchin egg cleavage. However, at $5 \times 10^{-5}M$ geldanamycin affects the MA in a manner analogous to that of maytansine and the effect is reversible.

Why some tumors should be sensitive to maytansine in vivo when it is an antimitotic agent which can also inhibit normal cells is not clear. We know of no evidence that tubulin of tumor cells differs from that of normal cells. However, microtubules have been implicated in certain cell surface related processes in lymphocytes, polymorphonuclear leukocytes, and other cells (*20*); and, since tumor cell surfaces differ from those of normal cells (*21*), it is not unlikely that specificity resides in such cell surface properties.

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**References and Notes**

1. S. M. Kupchan, Y. Komoda, W. A. Court, G. J. Thomas, R. M. Smith, A. Karim, C. J. Gilmore, R. C. Haltiwanger, R. F. Bryan, *J Am. Chem. Soc.* **95**, 1354 (1972).
2. S. M. Kupchan, *Fed. Proc.* **33**, 2288 (1974).
3. Maytansine is sparingly soluble in water. It was stored at 4°C at $3 \times 10^{-3}M$ in dimethyl sulfoxide and diluted into seawater or tissue culture media as desired; final concentrations of dimethyl sulfoxide were never above 0.1 percent. These levels had no effects on egg development, cell morphology, or cell division in tissue culture.
4. R. T. Hindgartner, B. Rad, D. E. Feldman, *Exp. Cell Res.* **36**, 53 (1964).
5. E. B. Wilson, *The Cell* (Macmillan, New York, 1928), p. 400.
6. L. I. Rebhun, M. Mellon, D. Jemiolo, J. Nath, N. Ivy, *J. Supramol. Struct.* **2**, 466 (1974); L. I. Rebhun, D. Jemiolo, N. Ivy, M. Mellon, J. Nath, *Ann. N.Y. Acad. Sci.*, in press.
7. R. D. Allen, *Biol. Bull. (Woods Hole)* **105**, 213 (1953); L. I. Rebhun, *ibid.* **117**, 518 (1959).
8. R. C. Weisenberg and A. C. Rosenfeld, *J. Cell Biol.* **64**, 146 (1975).
9. L. I. Rebhun and N. Sawada, *Protoplasma* **68**, 1 (1969).
10. R. C. Weisenberg, *Science* **177**, 1104 (1972).
11. L. I. Rebhun, *Am. Zool.*, in press.
12. F. Gaskin and C. R. Cantor, *J. Mol. Biol.* **89**, 737 (1974); Y. P. Lee, F. E. Samson, L. L. Houston, R. H. Himes, *J. Neurobiol.* **5**, 317 (1974).
13. W. D. Cohen and L. I. Rebhun, *J. Cell Sci.* **6**, 159 (1969).
14. M. K. Wolpert-Defilippes, R. H. Adamson, R. L. Cysyk, D. G. Jones, *Biochem. Pharmacol.* **24**, 751 (1975).
15. L. Wilson, J. R. Bamburg, S. B. Mizel, L. M. Grishom, K. M. Creswell, *Fed. Proc.* **33**, 158 (1974).
16. I. Cornman and M. E. Cornman, *Ann. N.Y. Acad. Sci.* **51**, 1443 (1951).
17. M. Mellon, J. Nath, L. I. Rebhun, unpublished results.
18. Maytanbutine 9-*n*-propyl thioether was obtained in 55 percent yield by treatment of maytanbutine in methylene chloride and trifluoroacetic acid with *n*-propyl thiol for 16 hours at room temperature. The physical constants for the thioether are: melting point (with decomposition) 202° to 204°C; specific rotation at 23°C for the sodium D line ($[\alpha]_D^{23}$) –97° (*c*, 0.0421, chloroform); ultraviolet absorption maximum (ethanol) (log $\epsilon$), 233 (4.41), 245 (4.38), 255 (4.41), 281 (3.71), 289 (3.69) nm; infrared absorption maximum (chloroform), 2.92, 3.34, 3.37, 3.41, 5.74, 5.86, 6.03, 6.12 μm; mass spectrum *m/e* 777 (M+); elemental analysis, found: C, 59.96 percent; H, 7.34 percent; N, 5.55 percent; calculated for $C_{39}H_{56}ClN_3O_9S$, C, 60.17 percent; H, 7.26 percent; N, 5.40 percent.
19. K. Sasaki, K. L. Rinehart, Jr., G. Slomp, M. F. Gnstic, E. C. Olsen, *J. Am. Chem. Soc.* **92**, 7591 (1970).
20. R. D. Berlin and T. E. Ukena, *Nat. New Biol.* **238**, 120 (1972); G. M. Edelman, I. Yahara, J. L. Wang, *Proc. Natl. Acad. Sci. U.S.A.* **70**, 1442 (1973); A. Hsie and T. T. Puck, *ibid.* **68**, 358 (1971).
21. R. Hynes, *Cell* **1**, 147 (1974).
22. Supported by NSF grant BMS 73-00812 A01 (to L.I.R.), NIH grant CA-12059 (to S.M.K.), and an NIH postdoctoral fellowship (G.A.H.). We thank Dr. J. Nath and M. Mellon for help with the tubulin polymerization experiments and Dr. H. B. Wood, Jr., for a sample of geldanamycin.

11 April 1975; revised 13 May 1975

## Cancer by County: New Resource for Etiologic Clues

Abstract. *Mapping of U.S. cancer mortality by county has revealed patterns of etiologic significance. The patterns for bladder cancer in males point to industrial determinants: some are known (chemical manufacturing) but others (automobile and machinery manufacturing) represent new leads for epidemiologic study. By contrast, the geographic clusters of high rates of stomach cancer in both sexes are consistent with ethnic susceptibility.*

Geographic variation in cancer mortality in the United States has usually been evaluated on a state-by-state basis. The paucity of clues to cancer etiology arising from such surveys can be traced to the heterogeneity of statewide populations. Counties may provide a compromise, as units small enough to be homogeneous for demographic and environmental characteristics that might influence cancer risk, and yet large enough for stable estimates of site-specific cancer mortality. We have made some preliminary observations that illustrate the value of county mortality measurements in providing leads to the origins of cancers.

We obtained age-, race-, and sex-specific numbers of cancer deaths for the 3056 counties of the contiguous United States over a 20-year period, 1950–1969, from the National Center for Health Statistics, Rockville, Maryland. Corresponding county populations were provided by the 1950, 1960, and 1970 censuses (*1*), with intercensal estimates derived by linear interpolation. For 35 cancer sites, we calculated age-standardized mortality rates by race and sex in each county, the standard being the age distribution of the entire U.S. population in 1960. Ninety-five percent confidence intervals were computed using the standard error of the age-standardized rate as determined by the method of Chiang (*2*). Differences between the county and national rates were statistically significant when the 95 percent confidence intervals for these rates did not overlap. Tabulations of cancer mortality rates by county were recently compiled (*3*).

Although population-based mortality data are a crude means of testing hypotheses concerning public health hazards, geographic correlations with environmental measurements can be done quickly and inexpensively, and may be a valuable first step in evaluating possible dangers. In this manner we have assessed cancer mortality patterns among people residing where drinking water is contaminated by asbestos (*4*), where homes are built on radioactive tailings from uranium mines (*5*), and where the chemical industry is highly concentrated (*6*).

The major contribution of the county resource, however, is in hypothesis formulation, namely the detection of geographic clustering that suggests etiologic clues, which can then be pursued by epidemiologic studies of an analytical type. Computer-generated maps were produced to visualize the spatial configuration of cancer mortality by county. We first plotted the distribution for bladder cancer, the tumor most strongly linked to occupational exposures (*7*). In white males there were clusters of elevated mortality in heavily industrialized areas (Fig. 1), a pattern that was not duplicated in females. The clusters in males suggest industrial hazards that should be evaluated.

To further characterize the possible hazards, we selected for correlation analysis a

Table 1. Industrial categories in which the percentage of men employed in counties where the bladder cancer risk is high differed significantly ($P < .05$) from the percentage of men employed nationwide. See text for method of selecting high-risk counties. Abbreviations: Exp., expected; Obs., observed.

| Industry | Percentage of employed men | | |
|---|---|---|---|
| | In the U.S. (Exp.) | In high-risk counties (Obs.) | Obs./Exp. |
| Agriculture | 15.8 | 4.2 | 0.3 |
| Mining | 2.2 | 0.3 | 0.1 |
| Manufacturing | 27.0 | 42.2 | 1.6 |
| Furniture, lumber, wood | 2.7 | 1.4 | 0.5 |
| Nonelectrical machinery | 2.8 | 6.3 | 2.3 |
| Electrical machinery | 1.3 | 2.8 | 2.2 |
| Motor vehicles | 1.9 | 4.8 | 2.5 |





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Exhibit 1029-34

# EXHIBIT E

*Cancer Treatment Reviews* (1978) 5, 199–207

# Maytansine

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## Introduction

Maytansine is a naturally occurring ansa macrolide with antitumor activity. It possesses metaphase arrest antimitotic properties which are also properties of the vinca alkaloids vincristine and vinblastine. Preclinical rodent tumor testing demonstrated high activity at very low dose levels and antitumor activity over a wide dose range. Phase I clinical testing by the National Cancer Institute (NCI) has now largely been completed and the compound is in Phase II trials.

The purpose of this paper is to review the available information on maytansine, especially with respect to an evaluation of its potential clinical usefulness.

## History

Maytansine was first isolated by Kupchan and coworkers (11, 12) in 1971 from alcoholic extracts of the East African shrub *Maytenus serrata* (formerly known as *M. ovatus*) and later from the wood and bark of *Maytenus buchananii*. It was the first ansa macrolide to be isolated from a plant rather than a micro-organism. Previously described ansa macrolides had demonstrated inhibition of bacterial DNA-dependent RNA polymerase (8, 17) and viral RNA-directed DNA polymerase (22), but maytansine was the first compound of this class to show significant antitumor activity (11, 12). It was found to be highly active against the mouse P388 lymphocytic leukemia and to also show activity against the L1210 mouse leukemia, the Lewis lung carcinoma and B-16 melanoma solid tumors (11, 12). Encouraged by its preclinical activity the NCI initiated Phase I clinical testing in 1976.

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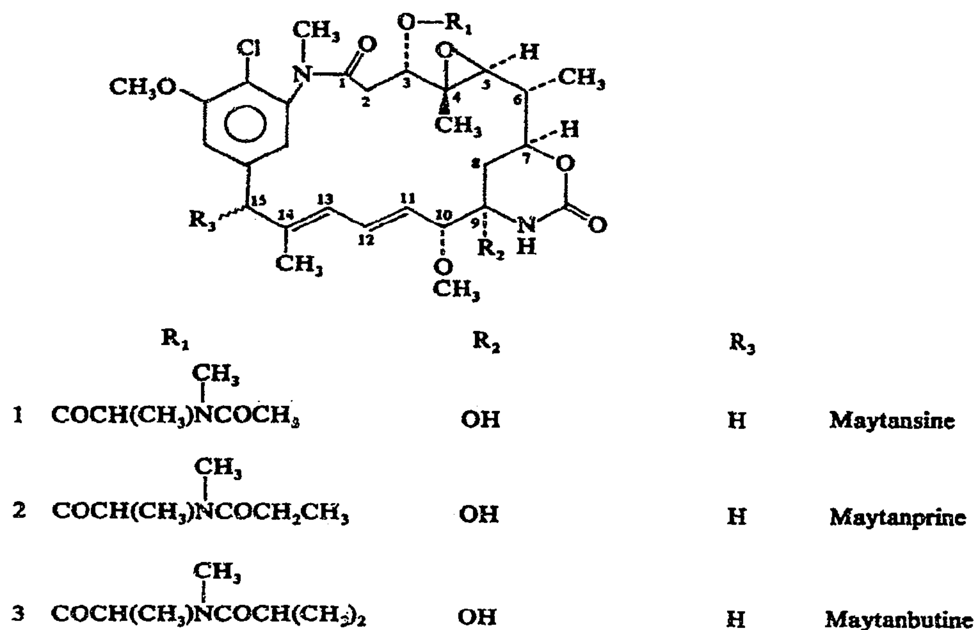
## Chemistry

The ansa macrolide class of compounds of which maytansine is a member includes the rifamycins and streptovarcins. The structural formula of maytansine is shown in Figure 1, and consists of an aromatic nucleus to which a macrocyclic aliphatic bridge is attached at two non-adjacent positions. Two homologue compounds are generally isolated with maytansine. These are maytanprine and maytanbutine and differ from maytansine by a methyl group in the first case and two methyl groups in the second case as shown in Figure 1. Both homologues have antitumor activity although to a lesser extent than maytansine in the P388 system (14). Maytansine can be differentiated from its homologues by chromatography in an ethyl acetate system on silica gel, using ultraviolet light to visualize the zones (7).



| | $R_1$ | $R_2$ | $R_3$ | |
|---|---|---|---|---|
| 1 | $COCH(CH_3)N(CH_3)COCH_3$ | OH | H | Maytansine |
| 2 | $COCH(CH_3)N(CH_3)COCH_2CH_3$ | OH | H | Maytanprine |
| 3 | $COCH(CH_3)N(CH_3)COCH(CH_3)_2$ | OH | H | Maytanbutine |

*Figure 1.* Structural formula of maytansine and homologues.

The structure activity relationships in the maytansinoid ansa macrolides have recently been reported for a small number of compounds (14). The carbinol amide and ester chain off C-3 appear to be necessary for significant antitumor activity (12, 14).

The extraction of maytansine from plant sources has resulted in low yields of active compound. A search for a microbiological source has recently been reported to be successful (10) and this new source will hopefully relieve the supply problems which have hindered the development of maytansine to date.

## Mechanism of action

Maytansine, like the vinca alkaloids vincristine and vinblastine, is a mitotic inhibitor. Treatment of L1210 cells *in vitro* with maytansine resulted in 67% of the cells accumulated in mitosis whereas the untreated control cells demonstrated a mitotic index ranging

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Exhibit 1029-37

between 3.2 and 5.8% (21). Flow microfluorimetry analysis of L1210 cells during exposure to maytansine indicated a shift in the distribution of DNA to a single peak, representing the DNA of cells in G2 & M Phases (23). Experiments with sea urchin eggs and clam eggs suggested that maytansine inhibited mitosis by interfering with the formation of microtubules by inhibiting the polymerization of the microtuble protein, tubulin (19).

The effects of maytansine at $10^{-7}$ M concentration on DNA, RNA and protein syntheses were examined in murine leukemia cell cultures (21, 22). DNA synthesis was inhibited to the greatest extent. In the P388 cells DNA synthesis was 14% of controls whereas RNA and protein syntheses were 46 and 48% of controls respectively. Unlike other ansa macrolides, maytansine did not inhibit *Escherichia coli* RNA polymerase activity at concentrations as high as $10^{-4}$ M (22).

As an antimitotic agent maytansine was found to be approximately 100 times more potent than vincristine in sea urchin eggs and 20 times more potent in Chinese Hamster ovary-K cells in tissue culture (20). However both drugs inhibited *in vitro* polymerization of tubulin at about the same concentrations (19). The differences in cellular activity between the two drugs may be explained by differences in uptake. In experiments with rat brain tubulin, maytansine and vincristine were found to bind reversibly and competitively (15). Both drugs were found to share a common binding site although an additional site specific for maytansine seemed to be present (15). The effects of maytansine and vincristine on the flow microfluorimetric characteristics of P388 murine leukemia *in vivo* have been compared. Similar cytokinetic effects were seen after the administration of both drugs although the effects were greater and more persistent with maytansine. Morphologically both drugs produced some degree of multinucleation and endoreduplication and vincristine also produced a population of cells with a DNA content, by fluorescence, equivalent to octaploidy.

## Preclinical activity

*In vitro* P388, L1210 and LY5178 murine leukemic cell suspensions were found to be inhibited by maytansine at doses of $10^{-3}$ to $10^{-7}$ μg/ml, with the P388 line being the most sensitive (21). Maytansine was shown to be an active inhibitor of *in vitro* growth of human nasopharyngeal carcinoma cells and the human lymphoblast leukemia line C.E.M. was inhibited by doses as low as $10^{-7}$ μg/ml (21).

Maytansine has also been shown to be active *in vivo* (21). The P388 lymphocytic leukemia system was inhibited over a 50- to 100-fold dosage range which suggested a high therapeutic index (11). Also maytansine was shown to have significant inhibitory activity against the L1210 mouse leukemia, the Lewis lung carcinoma and B-16 melanocarcinoma solid murine tumor systems (11). The optimal antitumor dose was 25 μg/kg/day for 10 consecutive days intraperitoneally for the P388, L1210 and B-16 tumor systems (21) and 32 μg/kg/day for 9 consecutive days for the Lewis lung carcinoma (9).

Maytansine treatment of mice inoculated with P388 cells intracerebrally resulted in only minimal antitumor activity and suggested that the drug does easily penetrate the blood–brain barrier in the mouse (21). In the P388 *in vivo* system maytansine was most active when given by a 3-hourly dosage schedule on Days 1, 5 and 9 (9). Maytansine was compared with vincristine *in vivo* and in vincristine-sensitive and resistant cell lines (22). Cross resistance was observed but maytansine was active against sensitive strains at a tenfold lower concentration than vincristine.

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Exhibit 1029-38

## Preclinical toxicity

### *Acute toxicity*

In the mouse the lethal dose in 10% of the animals treated ($LD_{10}$) was 1.22 $mg/m^2$ for males and 1.29 $mg/m^2$ for females when maytansine was given by intraperitoneal injection. Histopathologic evaluation of selected organs from the mice revealed lymphoid depletion of splenic follicles, fatty change and mild granular degeneration of hepatocvtes. No other drug related changes were observed (9).

In the rat after a single subcutaneous injection the $LD_{10}$ was of the same magnitude as for the mouse at 1.22 $mg/m^2$ (0.4 mg/kg). Histologically, necrotizing lesions were seen in the gastrointestinal tract mucosa, thymus, spleen, bone marrow and testes. Of considerable interest is the reported observation of hemorrhagic lesions of the brain, mononuclear infiltration in the meninges and chromatolysis and vacuolation of dorsal root ganglion cells (18).

In the beagle dog (9) the toxic dose low was 0.3 $mg/m^2$ when maytansine was given as a single intravenous dose and 0.75 $mg/m^2$ when divided over 5 daily administrations. In the Rhesus monkey the toxic dose low was 0.45 $mg/m^2$ when divided over 5 daily intravenous injections (9).

### *Chronic toxicity*

Multiple dose and more chronic treatment schedules in the beagle dog and monkey (9), resulted in pancreatic acinar cell degeneration and nephrosis. Increased mitotic activity was observed in numerous tissues including the pancreas, esophagus, stomach, small and large intestines, adrenal cortex, renal pelvis ureter, urinary bladder, and skin. The results from these studies suggested that toxicity from maytansine was dose related, reversible (except for histopathologic liver lesions) and non cumulative.

### *Neurotoxicity*

The neurotoxic effects of maytansine, vincristine and vinblastine were compared in mice by observing hind limb paralysis following administration of toxic doses (21). Vincristine was found to be neurotoxic causing 80 to 90% of mice to develop hind limb paralysis. In contrast vinblastine was not neurotoxic at the doses given and maytansine produced only mild hind limb paralysis in 10% of the mice receiving daily subcutaneous doses of 1.20 $mg/m^2$.

### *Teratogenicity*

Pregnant mice were treated with single injections of maytansine on Days 6, 7 and 8 of gestation and their fetuses examined for malformation of Day 17 of gestation (21). Both embryotoxic and teratogenic effects which appeared to be dose related were demonstrated. They were most marked when maytansine was administered on Day 7 of gestation.

### *Injection site*

When maytansine was given by subcutaneous injection in several animals a local tissue reaction with inflammation and fibrosis was observed (9).

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Exhibit 1029-39

## Pharmacokinetics

No satisfactory methodology has thus far been developed for detecting the low concentrations of maytansine present in human blood and tissues following dosage in the clinical range. A quantitative microbiological assay using *Penicillium avellaneum* OC-4376 has been described but the sensitivity of this assay is inadequate (7). The competitive displacement of $^3$H-vincristine by maytansine on rat brain tubulin (16), has been investigated as a quantitative assay of maytansine, but has yet to be proven effective. Chabner *et al.* (5) using this methodology found that the assay was insufficiently sensitive to measure the low serum levels of maytansine present at clinically tolerated doses.

The development of a radioimmunoassay has been hindered to date by an insufficient supply of maytansine to induce animal antibody production. The future supply of maytansine by a fermentation process (10) rather than by extraction of plants will hopefully allow quantities sufficient for radioimmunoassay development.

## Clinical experience

### *Maximum tolerated dose*

The maximum tolerated doses (MTD) generated from the National Cancer Institute Phase I and early Phase II trials are shown in Table 1. There was a good agreement among the dose levels reported from the contributing institutions. The MTD was in the 2 mg/m$^2$ range when maytansine was given every 3 to 4 weeks either as a single dose or divided over 3 daily doses. When given by weekly injections in the M.D. Anderson Phase II study (3) doses between 0.75 and 1.25 mg/m$^2$ were the maximum tolerated.

### *Toxicities*

*Gastrointestinal.* The most common and dose limiting toxicities were gastrointestinal and consisted primarily of nausea, vomiting and diarrhea, often followed by constipation. These toxicities appeared to be dose related.

**Table 1. Maximum tolerated doses of maytansine according to schedule**

| Institution (Reference) | Maximum tolerated dose (mg/m$^2$) | Schedule | Interval between course (days) | Dose limiting toxicity |
|---|---|---|---|---|
| Mayo Clinic (6) | 2.25 | Divided dose Days 1, 3, 5 | 28 | Gastrointestinal Weakness |
| National Cancer Institute (5) | 2.0 | Single dose Day 1 | 21 | Gastrointestinal |
| M.D. Anderson Hospital | | | | |
| Phase I (4) | 1.8–2.1 | Divided dose Day 1–3 | 21 | Gastrointestinal |
| Phase II (3) | 1.8 | Divided dose Day 1–3 | 14 | Gastrointestinal |
| Phase II (3) | 0.75–1.25 | Divided dose Day 1–3 | 7 | Gastrointestinal |
| Sidney Farber (2) | 2.0–2.5 | Divided dose Day 1–5 | 21 | Gastrointestinal |

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Exhibit 1029-40

In the M. D. Anderson experience reported by Cabanillas *et al.* (·
toxicities were first seen at a dose of 1.2 mg/m$^2$ (0.4 mg/m$^2$/day × 3 days) and consisted of nausea and vomiting which became progressively more severe at higher dose levels and lead to severe dehydration within a few hours.

Eagan *et al.* (6) from the Mayo Clinic reported that severe nausea and vomiting were usually observed in patients receiving doses of 1.8 mg/m$^2$ or greater. These toxicities generally began on or about Day 4 or 5 and persisted for 1 to 5 days. They were sometimes accompanied by abdominal cramps and watery diarrhea. Chabner *et al.* (5) at the NCI reported that 2 of 3 patients treated at a dose of 2 mg/m$^2$ developed severe diarrhea lasting 5 to 14 days. In the Sidney Farber experience reported by Blum *et al.* (2) nausea and diarrhea started around Day 3 and ended by Day 8. Patients also reported constipation during the second week of therapy. Other gastrointestinal related symptoms were anorexia and taste change lasting until the second week of therapy.

*Central nervous system.* Other incapacitating toxicities were considered to be due to the effect of maytansine on the central nervous system. These consisted of profound weakness (5), lethargy, dysphoria and insomnia (2). A decrease in performance status following maytansine administration was considered to be predominantly due to central toxicity (2). These toxicities appeared to be distinct from peripheral nervous system toxicities and also were not related to metabolic or electrolytic abnormalities (5). It is of interest that these severe incapacitating central nervous system toxicities were not reported in the M. D. Anderson and Mayo Clinic studies when the total maytansine dose was divided into 3 daily administrations. The central nervous system and gastrointestinal symptoms have been responsible for patients refusing further courses of therapy (5).

*Peripheral nervous system.* Dose limiting vincristine-like peripheral neuropathies have been reported after treatment with maytansine by Blum *et al.* (2). Patients complained of jaw pain and parasthesia as well as severe myalgia. The loss of deep tendon reflexes and marked prolongation of nerve conduction times have also been noted. Patients with prior neuropathy either secondary to malignancy or vincristine demonstrated further neurologic toxicity from maytansine. Transient parasthesia for 24 h following drug administration was also reported by Chabner *et al.* (5) in 4 patients and Cabanillas *et al.* (3) in a Phase II study reported parasthesia in 3 patients and adynamic ileus in 2 patients.

*Hepatic.* Transient elevations of serum transaminase, alkaline phosphatase and bilirubin levels have been reported in all the Phase I studies (2, 4–6). In patients without initial liver impairment these elevations returned to normal by Day 29. In the M. D. Anderson study 3 patients with prior abnormal hepatocellular function had rapid deterioration of liver function following maytansine therapy and subsequently died. However hepatic toxicity was considered to be the cause of death in only 1 patient (4).

*Myelosuppression.* Myelosuppression does not appear to be a dose limiting toxicity of maytansine. Thrombocytopenia was reported in less than 11% of courses in 2 studies and was found predominantly in patients with liver function abnormality (4, 6).

*Phlebitis.* Local minor phlebitis was reported in two studies. In one study it was eliminated by diluting the drug in larger volumes (250 to 500 ml) of fluid (4) and in the other by using rapid bolus injection (2).

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Exhibit 1029-41

**Table 2. Summary of tumors responsive to maytansine**

| Tumor type | Total evaluable patients | CR | PR | Improvement < PR | Duration of effect | Reference |
|---|---|---|---|---|---|---|
| Acute lymphoblastic leukemia | 4 | 1 | 1 | | 1 Adult with M1 remission for 1 month–1 child with clearing of marrow and peripheral blasts for 3½ months | (5) |
| Non-Hodgkins lymphoma | 3 | 1 | | | 9 months | (5) |
| Ovary | 5 | | 1 | | 5 months | (5) |
| Breast | 25 | | 1 | 2 | Partial response for 1 month | (2–6) |
| Melanoma | 27 | | 1 | 2 | Partial response for 5+ months | (2–6) |
| Head and neck | 2 | | | 1 | | (2,4) |

(CR = Complete response; PR = Partial response = Tumor regression ≥ 50%).

*Other.* Other infrequently reported toxicities included moderate stomatitis and mild alopecia (4).

*Antitumor activity*

The cumulative antitumor response reported in clinical studies is shown in Table 2. The responses in breast cancer and melanoma seen in their Phase I study (4) encouraged the M. D. Anderson group to conduct a Phase II study in these tumor types. However only 2 of 11 patients with melanoma had objective tumor regressions but these were less than 50%, and no objective responses were seen in the 6 breast cancer patients in this study (3).

The most encouraging antitumor activity was seen in patients with acute lympho-

**Table 3. Summary of evaluable tumor types without demonstrable objective response to maytansine**

| Tumor type | Number of evaluable patients treated | Reference |
|---|---|---|
| Acute non-lymphoblastic leukemia | 3 | (5) |
| Hodgkins lymphoma | 2 | (5) |
| Sarcoma | 9 | (2, 5, 6, ) |
| Colorectal | 27 | (2, 6) |
| Stomach | 2 | (6) |
| Pancreatic | 2 | (5, 6) |
| Adenocarcinoma unknown primary | 4 | (4, 6) |
| Hepatoma | 1 | (5) |
| Lung (including 1 specified small cell) | 12 | (2, 4, 6) |
| Prostate | 2 | (6) |
| Renal | 2 | (2, 4) |
| Thymoma | 1 | (6) |

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Exhibit 1029-42

blastic leukemia (ALL) where 2 of 4 patients responded including 1 complete remission and malignant lymphoma where 1 of 3 patients achieved a complete remission (5). These responses were seen in patients who had previously been treated with vincristine. One of the ALL patients was clearly clinically resistant to vincristine and the patient with malignant lymphoma had failed therapy with VP-16 and with a vincristine-containing combination.

A summary of tumor types not demonstrating any objective response to maytansine therapy is shown in Table 3. In all of these tumors with the possible exception of colorectal adenocarcinoma too few patients at sufficiently high concentrations of drug have been evaluated to state that maytansine is inactive. The activity of maytansine in small cell lung cancer is of special interest because of the responsiveness of this tumor to vincristine. However of the 12 evaluable lung cancer patients only 1 was specified as being of this histologic type.

## Summary and conclusions

1. Maytansine is an antitumor agent with antimitotic properties similar to the vinca alkaloids. Although its mechanism of action is similar to vincristine, preclinical studies suggest maytansine is more potent, and has an additional tubulin binding site. Maytansine may therefore be active in tumors resistant to the vinca alkaloids.
2. Of importance to the further clinical development of maytansine is the development of a quantitative assay with sufficient sensitivity to detect serum and tissue levels present at clinically tolerated doses. Present difficulties with drug supply will hopefully be relieved by the development of a fermentation process which in turn may allow sufficient quantities of drug for the development of a radioimmunoassay.
3. The findings of central nervous system toxicity in both animal and human studies suggests that maytansine may cross the blood–brain barrier and this should be clarified by the development of a quantitative assay to measure cerebrospinal fluid drug concentrations. Maytansine efficacy against brain tumors should be carefully evaluated in future studies.
4. The main dose limiting toxicities relate to the effects of maytansine on the gastrointestinal tract and nervous systems. The contribution of neurotoxicity to gastrointestinal toxicity is difficult to evaluate. It is possible that the excretion of maytansine and/or its metabolites into the gastrointertinal tract via the hepatobiliary pathway may be directly responsible for much of the gastrointestinal effects. If so the therapeutic manipulation to increase gastrointestinal transit at the time of drug administration or the prescribing of substances by mouth which will preferentially bind the drug and its toxic products in the gastrointestinal tract are potential methods for relieving gastrointestinal toxicity. An assay to measure fecal drug concentrations will help answer these questions.
5. The central nervous system toxicities which have been responsible for patients refusing further therapy in some instances are possible related to peak serum levels. This is suggested by these toxicities being reported only in studies in which the total maytansine dose was given as a single administration. Clearly further investigation is required to determine the optimal therapeutic index and the development of a drug assay will help achieve this objective.

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Exhibit 1029-43

6. The administration of maytansine, like vincristine is associated with peripheral neuropathy. This may be cumulative and will need to be carefully evaluated in the future.
7. Antitumor activity has been demonstrated in lymphoblastic leukemia and malignant lymphoma; malignancies which are also responsive to vincristine. The clinical data suggest that maytansine is active in these tumors after the development of vincristine resistance and should encourage a more thorough evaluation of this drug in malignancies of lymphoid origin. A careful evaluation of maytansine in small cell lung cancer, another vincristine responsive tumor, should also be undertaken.

## Acknowledgement

The authors wish to thank Ms Carol Boyd for typing this manuscript.

## References

1. Alabaster, O. & Cassidy, M. (1978) *J. natl. Cancer Inst.* **60:** 649–652.
2. Blum, R. H. & Kahlert, T. (1978) *Cancer Treat. Rep.* **62:** 435–438.
3. Cabanillas, F., Rodriguez, V. & Bodey, G. P. (1978) *Proc. AACR/ASCO* **19:** 102.
4. Cabanillas, F., Rodriguez, V., Hall, S. W., Burgess, M. A., Bodey, G. P. & Freireich, E. J. (1978) *Cancer Treat. Rep.* **62:** 425–428.
5. Chabner, B. A., Levine, A. S., Johnson, B. L. & Young, R. C. (1978) *Cancer Treat. Rep.* **62:** 429–433.
6. Eagan, R. T., Ingle, J. N., Robin, J., Frytak, S. & Moertel, C. G. (1978) *J. natl. Cancer Inst.* **60:** 93–96
7. Hanka, L. J. & Barnett, M. S. (1974) *Antimicrob. Ag. Chemother.* **6:** 651–652.
8. Hartmann, G., Hänikel, K. O., Knusel, F. & Nuesch, J. (1967) *Biochem. Biophys. Acta* **145:** 843.
9. Helman, L., Henney, J. & Slavik, M. (1976) *Maytansine (NSC 153858) Clinical Brochure.* Investigational Drug Branch, Division of Cancer Treatment, National Cancer Institute.
10. Higashide, E., Asai, M., Ootsu, K., Tanida, S., Kozai, Y., Hasegawa, T., Kishi, T., Sugino, Y. & Yonoa, M. (1977) *Nature* **270:** 721–722.
11. Kupchan, S. M. (1974) *Fed. Proc.* **33:** 2288–2295.
12. Kupchan, S. M., Komoda, Y., Branfman, A. R., Dailey, R. G. & Zimmerly, V. A. (1974) *J. Am. Chem. Soc.* **96:** 3706–3708.
13. Kupchan, S. M., Komoda, Y., Court, W. A., Tomas, G. J., Smith, R. M., Karim A., Gillmore, C. J., Haltiwanger, R. C. & Bryan, R. F. (1972) *J. Am. Chem. Soc.* **94:** 1354–1356.
14. Kupchan, S. M., Sneden, A. T., Branfman, A. R. & Howie, G. A. (1978) *J. Med. Chem.* **21:** 31–37
15. Mandelbaun-Shavit, F., Wolpert-DeFillippes, M. K. & Johns, D. G. (1976) *Biochem. Biophys. Res. Commun.* **72:** 47–54.
16. Mandelbaun-Shavit, Wolpert-DeFillippes, M. K., Johns, D. G. (1976) *Fed. Proc.* 35: 786.
17. Mizuno, S., Yamazaki, H., Nitta, K. & Unezawa, H. (1968) *Biochem. Biophys. Acta* **157:** 322.
18. Mugera, G. M. & Ward, G. M. (1977) *Cancer Treat. Rep.* **61:** 1333–1338.
19. Remillard, S. & Rebhun, L. I. (1975) *Science* **189:** 1002–1005.
20. Schaitman, T., Rebhun, L. I., and Kupchan, S. M. (1975) *J. Cell Biol.* **67:** 388a.
21. Sieber, S. M., Wolpert, M. K., Adamson, R. H., Cysyk, R. L., Bono, V. H. & Johns, D. G. (1976) *Comparative Leukemia Research 1975, Bibl. Heamat.* **43:** 495–500.
22. Wolpert-DeFillippes, M. K., Adamson, R. H., Cysyk, R. L., and Johns, D. G. (1975) *Biochem. Pharmacol.* **24:** 751–754.
23. Wolpert-DeFillippes, M. K., Bono, V. H., Dion, R. L. & Johns, D. G. (1975) *Biochem. Pharmacol.* **24:** 1735–1738.

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Exhibit 1029-44

# EXHIBIT F

# Monoclonal antibody drug conjugates in the treatment of cancer

Pamela A Trail* and Albert B Bianchi†

Monoclonal antibodies directed to tumor-associated antigens have been chemically conjugated to drugs with different mechanisms of action and different levels of potency. Monoclonal-antibody-directed drug delivery has the potential to both improve efficacy and reduce systemic toxicity. Several immunoconjugates have demonstrated impressive antigen-specific antitumor activity in preclinical models. Phase I trials of a calicheamicin immunoconjugate for treatment of acute myeloid leukemia and a doxorubicin immunoconjugate for treatment of carcinoma have recently been completed.

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**Abbreviations**

| | |
|---|---|
| **AML** | acute myeloid leukemia |
| **DOX** | doxorubicin |
| **MAb** | monoclonal antibody |
| **MR** | molar ratio |

## Introduction

The treatment of cancer is limited by a number of factors including the low therapeutic index of most chemotherapeutic agents, the emergence of drug- and radiation-resistant populations, tumor heterogeneity and the presence of metastatic disease. One of the means to improve the therapeutic index of drugs is by selective or 'targeted' delivery to tumor sites. Tumor-directed therapy has the potential to improve efficacy, by increasing the intratumoral concentration of the targeted agent, and to minimize toxicity by reducing systemic exposure. Monoclonal antibodies (MAbs), MAb fragments, hormones and growth factors have been used to deliver drugs, toxins, radionuclides, enzymes, photosensitizers and cytokines to tumors.

Unfortunately, the clinical efficacy of MAb-directed therapy is frequently limited by expression of the targeted antigen on normal as well as malignant cells. With the exception of MAbs to idiotypic domains of lymphocytes, truly tumor-specific MAbs have not been identified; rather, MAbs identify tumor-associated antigens expressed at higher density on malignant cells relative to normal cells. It is therefore necessary to balance the relative selectivity of the MAb with the potency of the agent delivered. Studies in preclinical models with human tumors in immunodeficient mice have demonstrated impressive activity for many of these conjugates. However, it is important to recognize that these models, while useful, frequently over-predict activity and under-predict toxicity because the antigen targeted is tumor-specific in the mouse but tumor-associated in patients. Nevertheless, several immunoconjugates have shown impressive activity even though the targeted antigen is expressed on normal tissues of immunodeficient animals or normal tissues of immunocompetent animals bearing syngeneic tumors [1,2,3•].

Significant progress has been made for MAb-directed therapies in the treatment of patients who have lymphoma [4,5••]. In addition, the use of the MAb 17-1A following tumor resection has resulted in improved survival of patients with Dukes' C colorectal cancer [6•] and improved response rates were seen in breast cancer patients receiving the anti-HER2 MAb, directed against the HER2 transmembrane tyrosine-kinase receptor coded by the *HER2* gene (also known as *neu* and as *c-erbB-2*), in combination with cisplatin [7]. However, only occasional responses have been reported for MAbs or immunoconjugates used as monotherapy in the treatment of patients with advanced solid tumors. The physical barriers of solid tumors — including elevated interstitial pressure, heterogeneous and reduced functional vasculature and the relatively large distances for MAbs to travel in the tumor interstitium [8•] — contribute to the limited tumor penetration and minimal efficacy seen when MAb-directed therapies are used as single agents in patients with advanced disease.

Several modifications have been used to improve the efficacy of immunoconjugate therapy. Immunogenicity has been reduced by using chimeric [9••] or humanized [5••] MAbs. Attempts to decrease the amount of MAb needed for antitumor activity have included the use of more potent drugs [3•,10] and alternative strategies such as branched linkers [11•] and delivery of liposome-encapsulated drugs [12,13•,14] to increase the quantity of drug delivered per antibody molecule. Improved MAb distribution in solid tumors has been addressed by using pharmacological approaches to improve penetration [15]. Recent studies have demonstrated that directing therapy to antigens expressed on the tumor vasculature (a readily accessible compartment), rather than to tumor-associated antigens of solid tumors, can produce impressive activity in preclinical models [16,17•,18••,19,20]. This review will concentrate on the results of recent clinical trials of MAb–drug immunoconjugates and highlight current strategies to improve the potency, specificity and efficacy of immunoconjugate therapy.

## MAb-directed delivery of enediynes

One means to improve immunoconjugate potency and efficacy is to increase the potency of the targeted drugs. Members of the enediyne family of antibiotics are among the

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Exhibit 1029-46

most toxic antitumor compounds described to date. This novel class of agents includes the calicheamicins, neocarzinostatin, esperamicins, dynemicins, kedarcidin and maduropeptin [21]. Although these agents are highly potent *in vitro*, their utility as antitumor drugs has — for the most part — been limited by their low therapeutic index. Antibody-directed delivery provides a potential means to exploit the impressive potency of these compounds while minimizing their systemic toxicity. The use of extremely toxic drugs requires careful MAb selection as even low levels of expression of the targeted antigen by normal cells may lead to significant toxicity. Neocarzinostatin [22–24] and several of the calicheamicins [3•,5••,10,25,26] have been used to produce extremely potent immunoconjugates.

The calicheamicins produce double-stranded breaks in DNA. Calicheamicin conjugates — in which a hydrazide of calicheamicin $\gamma^I_1$ was linked to oxidized sugars on the internalizing anti-polyepithelial-mucin MAb CT-M-01 — produced potent antigen-specific activity against subcutaneous breast-tumor xenografts in athymic mice [10].

A Phase I study of CMA-676, an immunoconjugate of calicheamicin $\gamma^I_1$ conjugated to a humanized (human $IgG_4$) anti-CD33 MAb (hP67.6), has recently been completed [5••]. The CD33 target antigen is expressed on acute myeloid leukemia (AML) and maturing hematopoietic cells but not on normal stem cells. Forty patients with refractory or relapsed AML were treated intravenously with 0.25–9.0 mg/m$^2$ of CMA-676. Toxicity was primarily hematologic; however neither the hematologic nor nonhematologic side effects was considered dose-limiting. Fever and chills occurred in 80% of patients and were the most common nonhematologic side effect. Leukemic cells were eliminated from the blood and marrow of 20% of treated patients. At the 9 mg/m$^2$ dose level, >75% saturation of CD33 sites was seen on peripheral-blood blast cells. Clinical responses were seen at dose levels of 1–9 mg/m$^2$. Responses were seen only in patients whose peripheral blast cell demonstrated ≥75% saturation of CD33 and had low efflux of 3,3′-diethyloxacarbocyanine iodide, an assay that determines functional efflux mediated through MDR1- and non-MDR1-dependent mechanisms. The efflux data suggest that intracellular delivery of the calicheamicin by the hP67.6 MAb did not overcome multidrug resistance. Data from this Phase I trial in patients with advanced AML are encouraging and support evaluation of CMA-676 in a setting of newly diagnosed or minimal-residual disease.

Calicheamicin $\theta^I_1$, a more potent analog of calicheamicin $\gamma^I_1$, was conjugated to an anti-ganglioside-$GD_2$ MAb (14G2a) and showed impressive antitumor activity when used to treat experimental liver metastases in syngeneic immunocompetent mice [3•]. Dose-dependent activity was observed against a neuroblastoma line heterogeneous for antigen expression. The conjugate of 14G2a with calichaemicin $\theta^I_1$ was both more efficacious and less toxic than unconjugated calicheamicin $\theta^I_1$ or mixtures of 14G2a and calicheamicin $\theta^I_1$, indicating effective antibody-directed targeting. The use of a syngeneic tumor model heterogeneous for antigen expression more closely approximates the clinical situation and provides an important model system for evaluating immunoconjugate efficacy.

## MAb-directed delivery of anthracyclines

The anthracycline family of antitumor antibiotics, most notably doxorubicin (DOX) and daunorubicin, has been used extensively for drug targeting applications [27]. The immunoconjugate BR96–DOX [1,28] was evaluated in Phase I [9••] and II [29•] clinical trials. BR96–DOX (which is chimeric with human $IgG_1$) binds a $Le^y$-related, tumor-associated antigen expressed on most human carcinomas [30] and on normal cells of the gastrointestinal tract of humans, dogs and rats [1]. BR96–DOX induced cures of human lung, breast and colon carcinomas in athymic mice and rats [1,27,31] and syngeneic colon tumors in immunocompetent rats [2].

Although cures were seen in multiple preclinical models, only tumor stabilization and a small number of partial regressions were seen in a Phase I trial of patients with advanced disease. A therapeutically relevant anticonjugate response was not observed and there were no significant hematologic or cardiac toxicities. The dose-limiting toxicity was acute gastrointestinal toxicity with dose-related nausea, vomiting and gastritis [9••].

A randomized Phase II trial was performed in patients with metastatic breast carcinoma [29•]. Patients received 700 mg/m$^2$ of BR96–DOX (20 mg/m$^2$ DOX) or 60 mg/m$^2$ of DOX every three weeks. There was one partial response (in a patient with hepatic metastases) in the fourteen patients receiving BR96–DOX and one complete and three partial responses in the nine patients receiving DOX. Interestingly, two of the four patients who crossed over to the BR96–DOX arm of the trial after persistent stable disease during DOX treatment achieved partial regression of hepatic metastases following BR96–DOX therapy.

Localization of BR96 and DOX was seen in tumor biopsies of patients receiving BR96–DOX, indicating that BR96 successfully delivered DOX to tumors [9••]. These data, taken together with the low clinical response rates, suggest that the dose that could be safely administered every three weeks was insufficient to maintain the intratumoral concentration of DOX required to achieve regression. Preclinical studies in antigen-expressing rats indicate that administering BR96–DOX in combination with cytotoxic drugs or at a low dose for an extended duration can substantially reduce both the dose per injection and the cumulative dose needed to cure established experimental tumors (PA Trail, unpublished data). It is likely that BR96–DOX, like most antibody therapies, will be most effective in minimal-disease settings.

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Exhibit 1029-47

Several novel conjugation strategies have been developed to improve the potency of anthracycline conjugates. An enzymatic coupling procedure that attaches DOX to galactose residues of an anti-carcinoembryonic-antigen MAb demonstrated antigen-specific activity *in vitro* and improved both the potency and efficacy (relative to unconjugated DOX) against tumors transplanted onto the chorioallantoic membrane of embryonated chicken eggs [32]. However, as conjugates were applied to tumor-containing discs on the chorioallantoic membrane, the relative utility of this method awaits demonstration of distal site activity in a more rigorous antitumor model that requires MAb-directed delivery.

The potency of immunoconjugates can be improved by increasing the quantity of drug delivered per MAb molecule. In the case of DOX immunoconjugates, significant losses in affinity and antigen-specific cytotoxicity were seen when $\geq$10 molecules of DOX were directly conjugated per MAb [33]. The use of branched linkers, in which each linker to the MAb carries two DOX molecules, resulted in an increase in the drug : MAb molar ratio (MR) from 8:1 to 16:1. This increase in the MR was accompanied by an increase in antigen-specific potency *in vitro* [11•] and a two-fold decrease in the amount of MAb required to achieve partial regression of subcutaneous tumors in preclinical models. The use of water-soluble polymeric carriers [34] has also been attempted to increase conjugate MRs.

## Immunoliposomes

The encapsulation of drugs in MAb-targeted liposomes can be used to selectively increase the concentration of drug delivered to antigen-expressing cells [12,13•,14,35]. The pharmacokinetics and clearance of liposomes were improved by incorporating lipid derivatives of polyethylene glycol (PEG) into liposome formulations [36,37]. These sterically stabilized liposomes enhance accumulation in tumours [38]. Importantly, immunoliposomes utilizing internalizing MAbs — such as anti-HER-2 [39] or anti-CD19 [13•] — can be used to selectively deliver high concentrations of drug into the cytoplasm of antigen-expressing cells.

## Targeting the tumor vasculature

The progressive growth and metastasis of tumors requires the formation of new blood vessels (angiogenesis) from the pre-existing vasculature [40]. Immunoconjugates directed against antigens differentially expressed on tumor endothelium offer several potential advantages over targeting tumor-associated antigens expressed on cells of solid tumors. Directing therapy to the accessible vascular compartment reduces the impact of the physical barriers of solid tumors, such as heterogeneous blood flow and elevated interstitial pressure, which restrict the penetration and distribution of MAbs through the tumor parenchyma [8•]. Endothelial cells are highly regulated, genetically stable cells that are less likely to develop the classical drug resistance observed in tumor cells [40]. In addition, as angiogenesis is required for tumor progression, therapies directed against the tumor vasculature should have broad-spectrum activity. Several recent studies have demonstrated that targeting the tumor vasculature with MAbs [16,17•,20], growth factor ligands [41,42] or peptides that bind $\alpha_v$ integrins [18••] can produce impressive antitumor activity. Identification of appropriate target antigens that are expressed on the tumor vasculature, but not on cells of normal vessels, is an area of ongoing interest. Potential antigens for vascular targeting include VEGFR-2 (vascular endothelial growth factor receptor 2), endoglin, endosialin, aminopeptidase A [16,19] and VEGF complexed with its receptor [43]. Screening of phage display libraries identified several peptides that selectively localized in the tumor vasculature. These peptides were conjugated to DOX and shown to have impressive antitumor activity that was associated with damage to the tumor vasculature [18••]. The *in vivo* screening of phage peptide libraries is an interesting approach to identify novel molecules expressed on angiogenic blood vessels.

## Conclusions and future directions

Although immunoconjugates are not currently established chemotherapeutic agents, several of them have demonstrated evidence of biologic activity in patients with advanced disease [5••,9••,29•]. The current objectives are aimed at improving the efficacy and therapeutic index of immunoconjugates by optimizing selectivity and potency. The development of MAb therapies directed against the tumor vasculature is an area of considerable interest and various research approaches to identify antigens and conjugation strategies with appropriate selectivity are being pursued.

The promise offered by MAb-based therapies has begun to be realized with the approval of an anti-CD20 MAb for treatment of non-Hodgkin's' lymphoma and an anti-HER2 MAb for treatment of metastatic breast carcinoma. The calicheamicin conjugate CMA-676 [5••] has shown encouraging data in a Phase I trial of patients with refractory AML. Although immunoconjugates may be active as single agents, it is likely that their major role — especially in treatment of solid tumors — will be in combination-chemotherapy regimens or minimal-disease settings. In addition to research efforts directed at improving immunoconjugate constructs, clinical studies to define optimal therapeutic strategies are underway and will further clarify the role of immunoconjugates as anticancer agents.

## References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

- • of special interest
- •• of outstanding interest

1. Trail PA, Willner D, Lasch SJ, Henderson AJ, Hofstead SJ, Casazza AM, Firestone RA, Hellström I, Hellström KE: **Cure of xenografted human carcinomas by BR96-doxorubicin immunoconjugates.** *Science* 1993, 261:212-215.

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Exhibit 1029-48

2. Sjögren HO, Isaksson M, Willner D, Hellström I, Hellström KE, Trail PA: **Antitumor activity of carcinoma-reactive BR96-doxorubicin conjugate against human carcinomas in athymic mice and rats and syngeneic rat carcinomas in immunocompetent rats.** *Cancer Res* 1997, **57**:4530-4536.

3. Lode HN, Reisfeld RA, Handgretinger R, Nicolaou KC, Gaedicke G,
• Wrasidlo W: **Targeted therapy with a novel enediyene antibiotic calicheamicin θ[I1] effectively suppresses growth and dissemination of liver metastases in a syngeneic model of murine neuroblastoma.** *Cancer Res* 1998, **58**:2925-2928.
This paper describes an anti-$GD_2$ MAb (anti-ganglioside) conjugated to a potent calicheamicin analogue. This immunoconjugate showed impressive antitumor activity in a minimal-disease liver metastasis model. This model has relevance to the clinical situation because a syngeneic tumor with heterogeneous expression of the $GD_2$ antigen was used to evaluate immunoconjugate activity.

4. Press OW, Eary JF, Appelbaum FR, Martin PJ, Badger CC, Nelp WB, Glenn S, Butchko G, Fisher D, Porter B *et al.*: **Radiolabeled-antibody therapy of B cell lymphoma with autologous bone marrow support.** *N Engl J Med* 1993, **329**:1219-1224.

5. Sievers EL, Appelbaum FR, Spielberger RT, Forman SJ, Flowers D,
•• Smith FO, Shannon-Dorcy K, Berger MS, Bernstein ID: **Selective ablation of acute myeloid leukemia using antibody-targeted chemotherapy: a phase I study of an anti-CD33 calicheamicin immunoconjugate.** *Blood* 1999, **93**:3678-3684.
This paper reports a phase I trial of an anti-CD33–calicheamicin conjugate, CMA-676, demonstrating a 20% response rate in patients with refractory AML. The study design included evaluation of the levels of saturation of CD33 sites and addressed the impact of a multi-drug-resistance phenotype on clinical response.

6. Riethmuller G, Holz E, Schlimok G, Schmiegel W, Raab R, Hoffken K,
• Gruber R, Funke I, Pichlmaier H, Hirche H *et al.*: **Monoclonal antibody therapy for resected Dukes' C colorectal cancer: seven-year outcome of a multicenter randomized trial.** *J Clin Oncol* 1998, **16**:1788-1794.
This study of 189 patients shows that 17-1A antibody, when used to treat minimal residual disease (post resection of Dukes' C colon carcinoma), prevented the development of distant metastasis in approximately one third of patients. The therapeutic effect was maintained after seven years of follow-up evaluation.

7. Pegram MD, Lipton A, Hayes DF, Weber BL, Baselga MM, Tripathy D, Baly D, Baighman SA, Twaddell T, Glaspy JA, Slalom DJ: **Phase II study of receptor-enhanced chemosensitivity using recombinant humanized anti-p185HER2/neu monoclonal antibody plus cisplatin in patients with HER2/neu-overexpressing metastatic breast cancer refractory to chemotherapy treatment.** *J Clin Oncol* 1998, **16**:2659-2663.

8. Jain RK: **Delivery of molecular and cellular medicine to solid**
• **tumors.** *J Control Release* 1998, **53**:49-67.
This reviews the physical barriers of solid tumors that impair the penetration, distribution and efficacy of MAb-directed therapies.

9. Saleh MN, LoBuglio AF, Trail PA: **Immunoconjugate therapy of**
•• **solid tumors: studies with BR96-doxorubicin.** In *Monoclonal Antibody-Based Therapy of Cancer.* Edited by Grossbard M. New York: Marcel Dekker, 1998:397-416.
This chapter discusses the preclinical biology and Phase I findings of BR96–DOX, an anticarcinoma immunoconjugate. Data from the Phase I trial demonstrate BR96-directed delivery of DOX in patient tumor biopsies and evidence of biologic activity. Low response rates indicate the need for further optimization for the conjugate to be clinically acceptable. It is suggested that BR96–DOX, like other MAb therapies, will be most effective when evaluated in minimal-disease settings.

10. Hinman LM, Hamann PR, Wallace R, Menendez AT, Durr FE, Upeslacis J: **Preparation and characterization of monoclonal antibody conjugates of the calicheamicins: a novel and potent family of antitumor antibiotics.** *Cancer Res* 1993, **53**:3336-3342.

11. King HD, Yurgaitis D, Willner D, Firestone RA, Yang MB, Lasch SJ,
• Hellstrom KE, Trail PA: **Monoclonal antibody conjugates of doxorubicin prepared with branched linkers: a novel method for increasing the potency of doxorubicin immunoconjugates.** *Bioconjug Chem* 1999, **10**:279-288.
The authors describe a novel conjugation strategy to increase immunoconjugate potency by increasing the amount of drug delivered per MAb. These linkers attach two DOX molecules per thiol to the MAb and the conjugates demonstrate potent antigen-specific cytotoxicity *in vitro.*

12. Park JW, Hong K, Kirpotin DB, Meyer O, Papahadjopoulos D, Benz CC: **Anti-HER2 immunoliposomes for targeted therapy of human tumors.** *Cancer Lett* 1997, **118**:153-160.

13. Lopes de Menezes DE, Pilarski LM, Allen TM: ***In vitro* and *in vivo***
• **targeting of immunoliposomal doxorubicin to human B-cell lymphoma.** *Cancer Res* 1998, **58**:3320-3330.
A study demonstrating the potential to use internalizing MAbs to deliver sterically stabilized DOX immunoliposomes in minimal-disease lymphoma models.

14. Tseng YL, Hong RL, Tao MH, Chang FH: **Sterically stabilized anti-idiotype immunoliposomes improve the therapeutic efficacy of doxorubicin in a murine B-cell lymphoma model.** *Int J Cancer* 1999, **80**:723-730.

15. Hornick JL, Khawli LA, Hu P, Sharifi J, Khanna C, Epstein AL: **Pretreatment with a monoclonal antibody/interleukin-2 fusion protein directed against DNA enhances the delivery of therapeutic molecules to solid tumors.** *Clin Cancer Res* 1999, **5**:51-60.

16. Huang X, Molema G, King S, Watkins L, Edgington TS, Thorpe PE: **Tumor infarction in mice by antibody-directed targeting of tissue factor to tumor vasculature.** *Science* 1997, **275**:547-550.

17. Ran S, Gao B, Duffy S, Watkins L, Rote N, Thorpe PE: **Infarction of**
• **solid Hodgkin's tumors in mice by antibody-directed targeting of tissue factor to tumor vasculature.** *Cancer Res* 1998, **58**:4646-4653.
This paper describes a strategy to inhibit tumor growth by selectively forming thrombi in the tumor vasculature. A MAb to a naturally occurring endothelial marker, vascular cell adhesion molecule 1 (VCAM-1), was used to target the extracellular domain of tissue factor.

18. Arap W, Pasqualini R, Ruoslahti E: **Cancer treatment by targeted**
•• **drug delivery to tumor vasculature in a mouse model.** *Science* 1998, **279**:377-380.
These studies use *in vivo* selection of phage display libraries to identify peptides that selectively localize in tumor blood vessels. This is an interesting approach to identify novel cell surface markers with selectivity for the tumor vasculature.

19. Arap W, Pasqualini R, Ruoslahti E: **Chemotherapy targeted to tumor vasculature.** *Curr Opin Oncol* 1998, **10**:560-565.

20. Matsuno F, Haruta Y, Kondo M, Tsai H, Barcos M, Seon BK: **Induction of lasting complete regression of preformed distinct solid tumors by targeting the tumor vasculature using two new anti-endoglin monoclonal antibodies.** *Clin Cancer Res* 1999, **5**:371-382.

21. Smith AL, Nicolaou KC: **The enediyne antibiotics.** *J Med Chem* 1996, **39**:2103-2117.

22. Kondo S, Nakatsu S, Sakahara H, Kobayashi H, Konishi J, Namba Y: **Antitumour activity of an immunoconjugate composed of anti-human astrocytoma monoclonal antibody and neocarzinostatin.** *Eur J Cancer* 1993, **3**:420-423.

23. Okamoto K, Yamaguchi T, Otsuji E, Yamaoka N, Yata Y, Tsuruta H, Kitamura K, Takahashi T: **Targeted chemotherapy in mice with peritoneally disseminated gastric cancer using monoclonal antibody-drug conjugate.** *Cancer Lett* 1998, **122**:231-236.

24. Otsuji E, Yamaguchi T, Tsuruta H, Yata Y, Nishi H, Okamoto K, Taniguchi K, Kato M, Kotani T, Kitamura K *et al.*: **The effect of intravenous and intra-tumoural chemotherapy using a monoclonal antibody-drug conjugate in a xenograft model of pancreatic cancer.** *Eur J Surg Oncol* 1995, **21**:61-65.

25. Schmidt CS, Wrasidlo W, Kaufmann O, Scherberich JE, Gaedicke G, Fischer P: **Monoclonal antibody 138H11 against gamma-glutamyltransferase provides a possible tool for targeting calicheamicin theta to renal cell carcinomas.** *Adv Exp Med Biol* 1998, **451**:431-436.

26. Siegel MM, Tabei K, Kunz A, Hollander IJ, Hamann RR, Bell DH, Berkenkamp S, Hillenkamp F: **Calicheamicin derivatives conjugated to monoclonal antibodies: determination of loading values and distributions by infrared and UV matrix-assisted laser desorption/ionization mass spectrometry and electrospray ionization mass spectrometry.** *Anal Chem* 1997, **69**:2716-2726.

27. Trail PA, Willner D, Hellstrom KE: **Site-directed delivery of anthracyclines for cancer therapy.** *Drug Dev Res* 1995, **34**:196-209.

28. Willner D, Trail PA, Hofstead SJ, King HD, Lasch SJ, Braslawsky GR, Greenfield RS, Kaneko T, Firestone RA: **(6-maleimidocaproyl)-hydrazone of doxorubicin - a new derivative for the preparation of immunoconjugates of doxorubicin.** *Bioconjugate Chem* 1993, 4:521-527.


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29. Tolcher AW, Sugarman S, Gelmon KA, Cohen R, Saleh M, Isaacs C,
• Young L, Healey D, Onetto N, Slichenmyer W: **Randomized phase II study of BR96-doxorubicin conjugate in patients with metastatic breast cancer.** *J Clin Oncol* 1999, **17**:478-484.
This article describes a Phase II clinical study of BR96–DOX in patients with metastatic breast cancer.

30. Hellström I, Garrigues HJ, Garrigues U, Hellström KE: **Highly tumor-reactive, internalizing, mouse monoclonal antibodies to Le$^{y}$-related cell surface antigen.** *Cancer Res* 1990, **50**:2183-2190.

31. Hellström I, Hellström KE, Siegall CB, Trail PA: **Immunoconjugates and immunotoxins for therapy of carcinomas.** In *Advances in Pharmacology*, vol 33, edn 1. Edited by August JT, Anders MW, Murad F, Coyle JT. San Diego: Academic Press; 1995:349-388.

32. Stan AC, Radu DL, Casares S, Bona CA, Brumeanu TD: **Antineoplastic efficacy of doxorubicin enzymatically assembled on galactose residues of a monoclonal antibody specific for the carcinoembryonic antigen.** *Cancer Res* 1999, **59**:115-121.

33. Shih LB, Goldenberg DM, Xuan H, Lu H, Sharkey RM, Hall TC: **Anthracycline immunoconjugates prepared by a site specific linkage via an amino-dextran intermediate carrier.** *Cancer Res* 1991, **51**:4192-4198.

34. Omelyanenko V, Gentry C, Kopeckova P, Kopecek J: **HPMA copolymer-anticancer drug-OV-TL16 antibody conjugates. II. Processing in epithelial ovarian carcinoma cells *in vitro*.** *Int J Cancer* 1998, **75**:600-608.

35. Crosasso P, Brusa P, Dosio F, Arpicco S, Pacchioni D, Schuber F, Cattel L: **Antitumoral activity of liposomes and immunoliposomes containing 5- fluorouridine prodrugs.** *J Pharm Sci* 1997, **86**:832-839.

36. Allen TM, Hansen C: **Pharmacokinetics of stealth versus conventional liposomes: effect of dose.** *Biochim Biophys Acta* 1991, **1068**:133-141.

37. Huwyler J, Yang J, Pardridge WM: **Receptor mediated delivery of daunomycin using immunoliposomes: pharmacokinetics and tissue distribution in the rat.** *J Pharmacol Exp Ther* 1997, **282**:1541-1546.

38. Hansen CB, Kao GY, Moase EH, Zalipsky S, Allen TM: **Attachment of antibodies to sterically stabilized liposomes: evaluation, comparison and optimization of coupling procedures.** *Biochim Biophys Acta* 1995, **1239**:133-144.

39. Kirpotin D, Park JW, Hong K, Zalipsky S, Li WL, Carter P, Benz CC, Papahadjopoulos D: **Sterically stabilized anti-HER2 immunoliposomes: design and targeting to human breast cancer cells *in vitro*.** *Biochemistry* 1997, **36**:66-75.

40. Folkman J: **New perspectives in clinical oncology from angiogenesis research.** *Eur J Cancer* 1996, **14**:2534-2539.

41. Olson TA, Mohanraj D, Roy S, Ramakrishnan S: **Targeting the tumor vasculature: inhibition of tumor growth by a vascular endothelial growth factor-toxin conjugate.** *Int J Cancer* 1997, **73**:865-870.

42. Arora N, Masood R, Zheng T, Cai J, Smith DL, Gill PS: **Vascular endothelial growth factor chimeric toxin is highly active against endothelial cells.** *Cancer Res* 1999, **59**:183-188.

43. Brekken RA, Huang X, King SW, Thorpe PE: **Vascular endothelial growth factor as a marker of tumor endothelium.** *Cancer Res* 1998, **58**:1952-1959.

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# EXHIBIT G

## HIGHLIGHTS OF PRESCRIBING INFORMATION

**These highlights do not include all the information needed to use TYKERB safely and effectively. See full prescribing information for TYKERB.**

**TYKERB (lapatinib) tablets**
**Initial U.S. Approval: 2007**

> **WARNING: HEPATOTOXICITY**
> *See full prescribing information for complete boxed warning.*
> **Hepatotoxicity has been observed in clinical trials and postmarketing experience. The hepatotoxicity may be severe and deaths have been reported. Causality of the deaths is uncertain.** *[See Warnings and Precautions (5.2).]*

### RECENT MAJOR CHANGES

| | |
|---|---|
| Indications and Usage. (1) | 01/2010 |
| Dosage and Administration. (2) | 01/2010 |
| Contraindications. (4) | 01/2010 |

### INDICATIONS AND USAGE

TYKERB, a kinase inhibitor, is indicated in combination with: (1)

- capecitabine, for the treatment of patients with advanced or metastatic breast cancer whose tumors overexpress HER2 and who have received prior therapy including an anthracycline, a taxane, and trastuzumab.
- letrozole for the treatment of postmenopausal women with hormone receptor positive metastatic breast cancer that overexpresses the HER2 receptor for whom hormonal therapy is indicated.

TYKERB in combination with an aromatase inhibitor has not been compared to a trastuzumab-containing chemotherapy regimen for the treatment of metastatic breast cancer.

### DOSAGE AND ADMINISTRATION

The recommended dosage of TYKERB for advanced or metastatic breast cancer is 1,250 mg (5 tablets) given orally once daily on Days 1-21 continuously in combination with capecitabine 2,000 mg/m$^2$/day (administered orally in 2 doses approximately 12 hours apart) on Days 1-14 in a repeating 21 day cycle. (2.1)

The recommended dose of TYKERB for hormone receptor positive, HER2 positive metastatic breast cancer is 1500 mg (6 tablets) given orally once daily continuously in combination with letrozole. When TYKERB is coadministered with letrozole, the recommended dose of letrozole is 2.5 mg once daily. (2.1)

- TYKERB should be taken at least one hour before or one hour after a meal. However, capecitabine should be taken with food or within 30 minutes after food. (2.1)
- TYKERB should be taken once daily. Do not divide daily doses of TYKERB. (2.1, 12.3)
- Modify dose for cardiac and other toxicities, severe hepatic impairment, and CYP3A4 drug interactions. (2.2)

### DOSAGE FORMS AND STRENGTHS

250 mg tablets (3)

### CONTRAINDICATIONS

Known severe hypersensitivity (e.g., anaphylaxis) to this product or any of its components. (4)

### WARNINGS AND PRECAUTIONS

- Decreases in left ventricular ejection fraction have been reported. Confirm normal LVEF before starting TYKERB and continue evaluations during treatment. (5.1)
- Lapatinib has been associated with hepatotoxicity. Monitor liver function tests before initiation of treatment, every 4 to 6 weeks during treatment, and as clinically indicated. Discontinue and do not restart TYKERB if patients experience severe changes in liver function tests. (5.2)
- Dose reduction in patients with severe hepatic impairment should be considered. (2.2, 5.3, 8.7)
- Diarrhea, including severe diarrhea, has been reported during treatment. Manage with anti-diarrheal agents, and replace fluids and electrolytes if severe. (5.4)
- Lapatinib has been associated with interstitial lung disease and pneumonitis. Discontinue TYKERB if patients experience severe pulmonary symptoms. (5.5)
- Lapatinib may prolong the QT interval in some patients. Consider ECG and electrolyte monitoring. (5.6, 12.6)
- Fetal harm can occur when administered to a pregnant woman. Women should be advised not to become pregnant when taking TYKERB. (5.7)

### ADVERSE REACTIONS

The most common (>20%) adverse reactions during treatment with TYKERB plus capecitabine were diarrhea, palmar-plantar erythrodysesthesia, nausea, rash, vomiting, and fatigue. The most common (≥20%) adverse reactions during treatment with TYKERB plus letrozole were diarrhea, rash, nausea, and fatigue. (6.1)

**To report SUSPECTED ADVERSE REACTIONS, contact GlaxoSmithKline at 1-888-825-5249 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch.**

### DRUG INTERACTIONS

- TYKERB is likely to increase exposure to concomitantly administered drugs which are metabolized by CYP3A4 or CYP2C8. (7.1)
- Avoid strong CYP3A4 inhibitors. If unavoidable, consider dose reduction of TYKERB in patients coadministered a strong CYP3A4 inhibitor. (2.2, 7.2)
- Avoid strong CYP3A4 inducers. If unavoidable, consider gradual dose increase of TYKERB in patients coadministered a strong CYP3A4 inducer. (2.2, 7.2)

**See 17 for PATIENT COUNSELING INFORMATION and FDA-approved patient labeling.**

**Revised: 01/2010**

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## FULL PRESCRIBING INFORMATION

**WARNING: HEPATOTOXICITY**

**Hepatotoxicity has been observed in clinical trials and postmarketing experience. The hepatotoxicity may be severe and deaths have been reported. Causality of the deaths is uncertain.** *[See Warnings and Precautions (5.2).]*

## 1 INDICATIONS AND USAGE

TYKERB® is indicated in combination with:

- capecitabine for the treatment of patients with advanced or metastatic breast cancer whose tumors overexpress HER2 and who have received prior therapy including an anthracycline, a taxane, and trastuzumab.
- letrozole for the treatment of postmenopausal women with hormone receptor positive metastatic breast cancer that overexpresses the HER2 receptor for whom hormonal therapy is indicated.

TYKERB in combination with an aromatase inhibitor has not been compared to a trastuzumab-containing chemotherapy regimen for the treatment of metastatic breast cancer.

## 2 DOSAGE AND ADMINISTRATION

### 2.1 Recommended Dosing

HER2 Positive Metastatic Breast Cancer: The recommended dose of TYKERB is 1,250 mg given orally once daily on Days 1-21 continuously in combination with capecitabine 2,000 $mg/m^2$/day (administered orally in 2 doses approximately 12 hours apart) on Days 1-14 in a repeating 21 day cycle. TYKERB should be taken at least one hour before or one hour after a meal. The dose of TYKERB should be once daily (5 tablets administered all at once); dividing the daily dose is not recommended *[see Clinical Pharmacology (12.3)]*. Capecitabine should be taken with food or within 30 minutes after food. If a day's dose is missed, the patient should not double the dose the next day. Treatment should be continued until disease progression or unacceptable toxicity occurs.

Hormone Receptor Positive, HER2 Positive Metastatic Breast Cancer: The recommended dose of TYKERB is 1,500 mg given orally once daily continuously in combination with letrozole. When coadministered with TYKERB, the recommended dose of letrozole is 2.5 mg once daily. TYKERB should be taken at least one hour before or one hour after a meal. The dose of TYKERB should be once daily (6 tablets administered all at once); dividing the daily dose is not recommended *[see Clinical Pharmacology (12.3)]*.

### 2.2 Dose Modification Guidelines

Cardiac Events: TYKERB should be discontinued in patients with a decreased left ventricular ejection fraction (LVEF) that is Grade 2 or greater by National Cancer Institute



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Common Terminology Criteria for Adverse Events (NCI CTCAE) and in patients with an LVEF that drops below the institution's lower limit of normal *[see Warnings and Precautions (5.1) and Adverse Reactions (6.1)]*. TYKERB in combination with capecitabine may be restarted at a reduced dose (1,000 mg/day) and in combination with letrozole may be restarted at a reduced dose of 1,250 mg/day after a minimum of 2 weeks if the LVEF recovers to normal and the patient is asymptomatic.

Hepatic Impairment: Patients with severe hepatic impairment (Child-Pugh Class C) should have their dose of TYKERB reduced. A dose reduction from 1,250 mg/day to 750 mg/day (HER2 positive metastatic breast cancer indication) or from 1,500 mg/day to 1,000 mg/day (hormone receptor positive, HER2 positive breast cancer indication) in patients with severe hepatic impairment is predicted to adjust the area under the curve (AUC) to the normal range and should be considered. However, there are no clinical data with this dose adjustment in patients with severe hepatic impairment.

Concomitant Strong CYP3A4 Inhibitors: The concomitant use of strong CYP3A4 inhibitors should be avoided (e.g., ketoconazole, itraconazole, clarithromycin, atazanavir, indinavir, nefazodone, nelfinavir, ritonavir, saquinavir, telithromycin, voriconazole). Grapefruit may also increase plasma concentrations of lapatinib and should be avoided. If patients must be coadministered a strong CYP3A4 inhibitor, based on pharmacokinetic studies, a dose reduction to 500 mg/day of lapatinib is predicted to adjust the lapatinib AUC to the range observed without inhibitors and should be considered. However, there are no clinical data with this dose adjustment in patients receiving strong CYP3A4 inhibitors. If the strong inhibitor is discontinued, a washout period of approximately 1 week should be allowed before the lapatinib dose is adjusted upward to the indicated dose. *[See Drug Interactions (7.2).]*

Concomitant Strong CYP3A4 Inducers: The concomitant use of strong CYP3A4 inducers should be avoided (e.g., dexamethasone, phenytoin, carbamazepine, rifampin, rifabutin, rifapentin, phenobarbital, St. John's Wort). If patients must be coadministered a strong CYP3A4 inducer, based on pharmacokinetic studies, the dose of lapatinib should be titrated gradually from 1,250 mg/day up to 4,500 mg/day (HER2 positive metastatic breast cancer indication) or from 1,500 mg/day up to 5,500 mg/day (hormone receptor positive, HER2 positive breast cancer indication) based on tolerability. This dose of lapatinib is predicted to adjust the lapatinib AUC to the range observed without inducers and should be considered. However, there are no clinical data with this dose adjustment in patients receiving strong CYP3A4 inducers. If the strong inducer is discontinued the lapatinib dose should be reduced to the indicated dose. *[See Drug Interactions (7.2).]*

Other Toxicities: Discontinuation or interruption of dosing with TYKERB may be considered when patients develop ≥Grade 2 NCI CTCAE toxicity and can be restarted at 1,250 mg/day when the toxicity improves to Grade 1 or less. If the toxicity recurs, then TYKERB in combination with capecitabine should be restarted at a lower dose (1,000 mg/day) and in combination with letrozole should be restarted at a lower dose of 1,250 mg/day.

**See manufacturer's prescribing information for the coadministered product dosage**



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**adjustment guidelines in the event of toxicity and other relevant safety information or contraindications.**

## 3 DOSAGE FORMS AND STRENGTHS

250 mg tablets — oval, biconvex, orange, film-coated with GS XJG debossed on one side.

## 4 CONTRAINDICATIONS

TYKERB is contraindicated in patients with known severe hypersensitivity (e.g., anaphylaxis) to this product or any of its components.

## 5 WARNINGS AND PRECAUTIONS

### 5.1 Decreased Left Ventricular Ejection Fraction

TYKERB has been reported to decrease LVEF *[see Adverse Reactions (6.1)]*. In clinical trials, the majority (>57%) of LVEF decreases occurred within the first 12 weeks of treatment; however, data on long-term exposure are limited. Caution should be taken if TYKERB is to be administered to patients with conditions that could impair left ventricular function. LVEF should be evaluated in all patients prior to initiation of treatment with TYKERB to ensure that the patient has a baseline LVEF that is within the institution's normal limits. LVEF should continue to be evaluated during treatment with TYKERB to ensure that LVEF does not decline below the institution's normal limits *[see Dosage and Administration (2.2)]*.

### 5.2 Hepatotoxicity

Hepatotoxicity (ALT or AST >3 times the upper limit of normal and total bilirubin >2 times the upper limit of normal) has been observed in clinical trials (<1% of patients) and postmarketing experience. The hepatotoxicity may be severe and deaths have been reported. Causality of the deaths is uncertain. The hepatotoxicity may occur days to several months after initiation of treatment. Liver function tests (transaminases, bilirubin, and alkaline phosphatase) should be monitored before initiation of treatment, every 4 to 6 weeks during treatment, and as clinically indicated. If changes in liver function are severe, therapy with TYKERB should be discontinued and patients should not be retreated with TYKERB *[see Adverse Reactions (6.1)]*.

### 5.3 Patients with Severe Hepatic Impairment

If TYKERB is to be administered to patients with severe pre-existing hepatic impairment, dose reduction should be considered *[see Dosage and Administration (2.2) and Use in Specific Populations (8.7)]*. In patients who develop severe hepatotoxicity while on therapy, TYKERB should be discontinued and patients should not be retreated with TYKERB *[see Warnings and Precautions (5.2)]*.

### 5.4 Diarrhea

Diarrhea, including severe diarrhea, has been reported during treatment with TYKERB *[see Adverse Reactions (6.1)]*. Proactive management of diarrhea with anti-diarrheal agents is important. Severe cases of diarrhea may require administration of oral or intravenous electrolytes and fluids, and interruption or discontinuation of therapy with TYKERB.



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### 5.5 Interstitial Lung Disease/Pneumonitis

Lapatinib has been associated with interstitial lung disease and pneumonitis in monotherapy or in combination with other chemotherapies *[see Adverse Reactions (6.1)]*. Patients should be monitored for pulmonary symptoms indicative of interstitial lung disease or pneumonitis. TYKERB should be discontinued in patients who experience pulmonary symptoms indicative of interstitial lung disease/pneumonitis which are ≥Grade 3 (NCI CTCAE).

### 5.6 QT Prolongation

QT prolongation was observed in an uncontrolled, open-label dose escalation study of lapatinib in advanced cancer patients *[see Clinical Pharmacology (12.4)]*. Lapatinib should be administered with caution to patients who have or may develop prolongation of QTc. These conditions include patients with hypokalemia or hypomagnesemia, with congenital long QT syndrome, patients taking anti-arrhythmic medicines or other medicinal products that lead to QT prolongation, and cumulative high-dose anthracycline therapy. Hypokalemia or hypomagnesemia should be corrected prior to lapatinib administration.

### 5.7 Use in Pregnancy

TYKERB can cause fetal harm when administered to a pregnant woman. Based on findings in animals, TYKERB is expected to result in adverse reproductive effects. Lapatinib administered to rats during organogenesis and through lactation led to death of offspring within the first 4 days after birth *[see Use in Specific Populations (8.1)]*.

There are no adequate and well-controlled studies with TYKERB in pregnant women. Women should be advised not to become pregnant when taking TYKERB. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

## 6 ADVERSE REACTIONS

### 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.

<u>HER2 Positive Metastatic Breast Cancer:</u> The safety of TYKERB has been evaluated in more than 12,000 patients in clinical trials. The efficacy and safety of TYKERB in combination with capecitabine in breast cancer was evaluated in 198 patients in a randomized, Phase 3 trial. *[See Clinical Studies (14.1).]* Adverse reactions which occurred in at least 10% of patients in either treatment arm and were higher in the combination arm are shown in Table 1.

The most common adverse reactions (>20%) during therapy with TYKERB plus capecitabine were gastrointestinal (diarrhea, nausea, and vomiting), dermatologic (palmar-plantar erythrodysesthesia and rash), and fatigue. Diarrhea was the most common adverse reaction resulting in discontinuation of study medication.

The most common Grade 3 and 4 adverse reactions (NCI CTCAE v3) were diarrhea and palmar-plantar erythrodysesthesia. Selected laboratory abnormalities are shown in Table 2.



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Exhibit 1029-56

**Table 1. Adverse Reactions Occurring in ≥10% of Patients**

| | TYKERB 1,250 mg/day + Capecitabine 2,000 mg/m²/day (N = 198) | | | Capecitabine 2,500 mg/m²/day (N = 191) | | |
|---|---|---|---|---|---|---|
| | All Grades[a] | Grade 3 | Grade 4 | All Grades[a] | Grade 3 | Grade 4 |
| **Reactions** | % | % | % | % | % | % |
| **Gastrointestinal disorders** | | | | | | |
| Diarrhea | 65 | 13 | 1 | 40 | 10 | 0 |
| Nausea | 44 | 2 | 0 | 43 | 2 | 0 |
| Vomiting | 26 | 2 | 0 | 21 | 2 | 0 |
| Stomatitis | 14 | 0 | 0 | 11 | <1 | 0 |
| Dyspepsia | 11 | <1 | 0 | 3 | 0 | 0 |
| **Skin and subcutaneous tissue disorders** | | | | | | |
| Palmar-plantar erythrodysesthesia | 53 | 12 | 0 | 51 | 14 | 0 |
| Rash[b] | 28 | 2 | 0 | 14 | 1 | 0 |
| Dry skin | 10 | 0 | 0 | 6 | 0 | 0 |
| **General disorders and administrative site conditions** | | | | | | |
| Mucosal inflammation | 15 | 0 | 0 | 12 | 2 | 0 |
| **Musculoskeletal and connective tissue disorders** | | | | | | |
| Pain in extremity | 12 | 1 | 0 | 7 | <1 | 0 |
| Back pain | 11 | 1 | 0 | 6 | <1 | 0 |
| **Respiratory, thoracic, and mediastinal disorders** | | | | | | |
| Dyspnea | 12 | 3 | 0 | 8 | 2 | 0 |
| **Psychiatric disorders** | | | | | | |
| Insomnia | 10 | <1 | 0 | 6 | 0 | 0 |

[a] National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.

[b] Grade 3 dermatitis acneiform was reported in <1% of patients in TYKERB plus capecitabine group.



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Exhibit 1029-57

**Table 2. Selected Laboratory Abnormalities**

| | TYKERB 1,250 mg/day + Capecitabine 2,000 mg/m²/day | | | Capecitabine 2,500 mg/m²/day | | |
|---|---|---|---|---|---|---|
| | All Grades[a] | Grade 3 | Grade 4 | All Grades[a] | Grade 3 | Grade 4 |
| **Parameters** | % | % | % | % | % | % |
| **Hematologic** | | | | | | |
| Hemoglobin | 56 | <1 | 0 | 53 | 1 | 0 |
| Platelets | 18 | <1 | 0 | 17 | <1 | <1 |
| Neutrophils | 22 | 3 | <1 | 31 | 2 | 1 |
| **Hepatic** | | | | | | |
| Total Bilirubin | 45 | 4 | 0 | 30 | 3 | 0 |
| AST | 49 | 2 | <1 | 43 | 2 | 0 |
| ALT | 37 | 2 | 0 | 33 | 1 | 0 |

[a] National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.

Hormone Receptor Positive, Metastatic Breast Cancer: In a randomized clinical trial of patients (N = 1,286) with hormone receptor positive, metastatic breast cancer, who had not received chemotherapy for their metastatic disease, patients received letrozole with or without TYKERB. In this trial, the safety profile of TYKERB was consistent with previously reported results from trials of TYKERB in the advanced or metastatic breast cancer population. Adverse reactions which occurred in at least 10% of patients in either treatment arm and were higher in the combination arm are shown in Table 3. Selected laboratory abnormalities are shown in Table 4.



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Exhibit 1029-58

**Table 3. Adverse Reactions Occurring in ≥10% of Patients**

| | TYKERB 1,500 mg/day + Letrozole 2.5 mg/day (N = 654) | | | Letrozole 2.5 mg/day (N = 624) | | |
|---|---|---|---|---|---|---|
| | All Grades[a] | Grade 3 | Grade 4 | All Grades[a] | Grade 3 | Grade 4 |
| **Reactions** | % | % | % | % | % | % |
| **Gastrointestinal disorders** | | | | | | |
| Diarrhea | 64 | 9 | <1 | 20 | <1 | 0 |
| Nausea | 31 | <1 | 0 | 21 | <1 | 0 |
| Vomiting | 17 | 1 | <1 | 11 | <1 | <1 |
| Anorexia | 11 | <1 | 0 | 9 | <1 | 0 |
| **Skin and subcutaneous tissue disorders** | | | | | | |
| Rash[b] | 44 | 1 | 0 | 13 | 0 | 0 |
| Dry skin | 13 | <1 | 0 | 4 | 0 | 0 |
| Alopecia | 13 | <1 | 0 | 7 | 0 | 0 |
| Pruritus | 12 | <1 | 0 | 9 | <1 | 0 |
| Nail Disorder | 11 | <1 | 0 | <1 | 0 | 0 |
| **General disorders and administrative site conditions** | | | | | | |
| Fatigue | 20 | 2 | 0 | 17 | <1 | 0 |
| Asthenia | 12 | <1 | 0 | 11 | <1 | 0 |
| **Nervous system disorders** | | | | | | |
| Headache | 14 | <1 | 0 | 13 | <1 | 0 |
| **Respiratory, thoracic, and mediastinal disorders** | | | | | | |
| Epistaxis | 11 | <1 | 0 | 2 | <1 | 0 |

[a] National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.

[b] In addition to the rash reported under "Skin and subcutaneous tissue disorders", 3 additional subjects in each treatment arm had rash under "Infections and infestations"; none were Grade 3 or 4.



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**Table 4. Selected Laboratory Abnormalities**

| | TYKERB 1,500 mg/day + Letrozole 2.5 mg/day | | | Letrozole 2.5 mg/day | | |
|---|---|---|---|---|---|---|
| | All Grades[a] | Grade 3 | Grade 4 | All Grades[a] | Grade 3 | Grade 4 |
| **Hepatic Parameters** | % | % | % | % | % | % |
| AST | 53 | 6 | 0 | 36 | 2 | <1 |
| ALT | 46 | 5 | <1 | 35 | 1 | 0 |
| Total Bilirubin | 22 | <1 | <1 | 11 | 1 | <1 |

[a] National Cancer Institute Common Terminology Criteria for Adverse Events, version 3.

Decreases in Left Ventricular Ejection Fraction: Due to potential cardiac toxicity with HER2 (ErbB2) inhibitors, LVEF was monitored in clinical trials at approximately 8-week intervals. LVEF decreases were defined as signs or symptoms of deterioration in left ventricular cardiac function that are ≥Grade 3 (NCI CTCAE), or a ≥20% decrease in left ventricular cardiac ejection fraction relative to baseline which is below the institution's lower limit of normal. Among 198 patients who received TYKERB/capecitabine combination treatment, 3 experienced Grade 2 and one had Grade 3 LVEF adverse reactions (NCI CTCAE v3). *[See Warnings and Precautions (5.1).]* Among 654 patients who received TYKERB/letrozole combination treatment, 26 patients experienced Grade 1 or 2 and 6 patients had Grade 3 or 4 LVEF adverse reactions.

Hepatotoxicity: TYKERB has been associated with hepatotoxicity *[see Boxed Warning and Warnings and Precautions (5.2)]*.

Interstitial Lung Disease/Pneumonitis: TYKERB has been associated with interstitial lung disease and pneumonitis in monotherapy or in combination with other chemotherapies *[see Warnings and Precautions (5.5)]*.

## 6.2 Postmarketing Experience

The following adverse reactions have been identified during post-approval use of TYKERB. 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.

Immune System Disorders: Hypersensitivity reactions including anaphylaxis *[see Contraindications (4)]*.

Skin and Subcutaneous Tissue Disorders: Nail disorders including paronychia.

# 7 DRUG INTERACTIONS

## 7.1 Effects of Lapatinib on Drug Metabolizing Enzymes and Drug Transport Systems

Lapatinib inhibits CYP3A4 and CYP2C8 in vitro at clinically relevant concentrations. Caution should be exercised and dose reduction of the concomitant substrate drug should be considered when dosing lapatinib concurrently with medications with narrow therapeutic windows that are substrates of CYP3A4 or CYP2C8. Lapatinib did not significantly inhibit the



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Exhibit 1029-60

following enzymes in human liver microsomes: CYP1A2, CYP2C9, CYP2C19, and CYP2D6 or UGT enzymes in vitro, however, the clinical significance is unknown.

Lapatinib inhibits human P-glycoprotein. If TYKERB is administered with drugs that are substrates of P-gp, increased concentrations of the substrate drug are likely, and caution should be exercised.

Paclitaxel: In cancer patients receiving TYKERB and the CYP2C8 substrate paclitaxel, 24-hour systemic exposure (AUC) of paclitaxel was increased 23%. This increase in paclitaxel exposure may have been underestimated from the in vivo evaluation due to study design limitations.

## 7.2 Drugs that Inhibit or Induce Cytochrome P450 3A4 Enzymes

Lapatinib undergoes extensive metabolism by CYP3A4, and concomitant administration of strong inhibitors or inducers of CYP3A4 alter lapatinib concentrations significantly *(see Ketoconazole and Carbamazepine sections, below)*. Dose adjustment of lapatinib should be considered for patients who must receive concomitant strong inhibitors or concomitant strong inducers of CYP3A4 enzymes *[see Dosage and Administration (2.2)]*.

Ketoconazole: In healthy subjects receiving ketoconazole, a CYP3A4 inhibitor, at 200 mg twice daily for 7 days, systemic exposure (AUC) to lapatinib was increased to approximately 3.6-fold of control and half-life increased to 1.7-fold of control.

Carbamazepine: In healthy subjects receiving the CYP3A4 inducer, carbamazepine, at 100 mg twice daily for 3 days and 200 mg twice daily for 17 days, systemic exposure (AUC) to lapatinib was decreased approximately 72%.

## 7.3 Drugs that Inhibit Drug Transport Systems

Lapatinib is a substrate of the efflux transporter P-glycoprotein (P-gp, ABCB1). If TYKERB is administered with drugs that inhibit P-gp, increased concentrations of lapatinib are likely, and caution should be exercised.

# 8 USE IN SPECIFIC POPULATIONS

## 8.1 Pregnancy

**Pregnancy Category D** ***[see Warnings and Precautions (5.7)].***

Based on findings in animals, TYKERB can cause fetal harm when administered to a pregnant woman. Lapatinib administered to rats during organogenesis and through lactation led to death of offspring within the first 4 days after birth. When administered to pregnant animals during the period of organogenesis, lapatinib caused fetal anomalies (rats) or abortions (rabbits) at maternally toxic doses. There are no adequate and well-controlled studies with TYKERB in pregnant women. Women should be advised not to become pregnant when taking TYKERB. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

In a study where pregnant rats were dosed with lapatinib during organogenesis and through lactation, at a dose of 120 mg/kg/day (approximately 6.4 times the human clinical exposure based on AUC following 1,250 mg dose of lapatinib plus capecitabine), 91% of the



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Exhibit 1029-61

pups had died by the fourth day after birth, while 34% of the 60 mg/kg/day pups were dead. The highest no-effect dose for this study was 20 mg/kg/day (approximately equal to the human clinical exposure based on AUC).

Lapatinib was studied for effects on embryo-fetal development in pregnant rats and rabbits given oral doses of 30, 60, and 120 mg/kg/day. There were no teratogenic effects; however, minor anomalies (left-sided umbilical artery, cervical rib, and precocious ossification) occurred in rats at the maternally toxic dose of 120 mg/kg/day (approximately 6.4 times the human clinical exposure based on AUC following 1,250 mg dose of lapatinib plus capecitabine). In rabbits, lapatinib was associated with maternal toxicity at 60 and 120 mg/kg/day (approximately 0.07 and 0.2 times the human clinical exposure, respectively, based on AUC following 1,250 mg dose of lapatinib plus capecitabine) and abortions at 120 mg/kg/day. Maternal toxicity was associated with decreased fetal body weights and minor skeletal variations.

### 8.3 Nursing Mothers

It is not known whether lapatinib is excreted in human milk. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from TYKERB, a decision should be made whether to discontinue nursing or to discontinue the drug, taking into account the importance of the drug to the mother.

### 8.4 Pediatric Use

The safety and effectiveness of TYKERB in pediatric patients have not been established.

### 8.5 Geriatric Use

Of the total number of metastatic breast cancer patients in clinical studies of TYKERB in combination with capecitabine (N = 198), 17% were 65 years of age and older, and 1% were 75 years of age and older. Of the total number of hormone receptor positive, HER2 positive metastatic breast cancer patients in clinical studies of TYKERB in combination with letrozole (N = 642), 44% were 65 years of age and older, and 12% were 75 years of age and older. No overall differences in safety or effectiveness were observed between elderly subjects and younger subjects, and other reported clinical experience has not identified differences in responses between the elderly and younger patients, but greater sensitivity of some older individuals cannot be ruled out.

### 8.6 Renal Impairment

Lapatinib pharmacokinetics have not been specifically studied in patients with renal impairment or in patients undergoing hemodialysis. There is no experience with TYKERB in patients with severe renal impairment. However, renal impairment is unlikely to affect the pharmacokinetics of lapatinib given that less than 2% (lapatinib and metabolites) of an administered dose is eliminated by the kidneys.

### 8.7 Hepatic Impairment

The pharmacokinetics of lapatinib were examined in subjects with pre-existing moderate (n = 8) or severe (n = 4) hepatic impairment (Child-Pugh Class B/C, respectively) and in 8 healthy control subjects. Systemic exposure (AUC) to lapatinib after a single oral 100-mg dose



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Exhibit 1029-62

increased approximately 14% and 63% in subjects with moderate and severe pre-existing hepatic impairment, respectively. Administration of TYKERB in patients with severe hepatic impairment should be undertaken with caution due to increased exposure to the drug. A dose reduction should be considered for patients with severe pre-existing hepatic impairment *[see Dosage and Administration (2.2)]*. In patients who develop severe hepatotoxicity while on therapy, TYKERB should be discontinued and patients should not be retreated with TYKERB *[see Warnings and Precautions (5.2)]*.

## 10 OVERDOSAGE

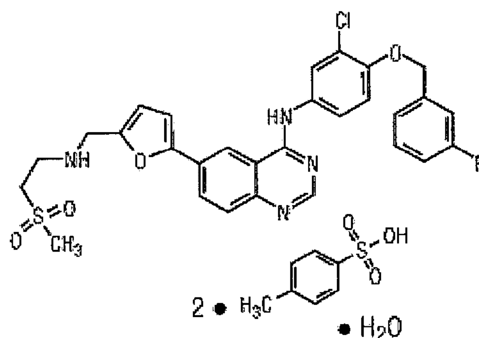
There is no known antidote for overdoses of TYKERB. The maximum oral doses of lapatinib that have been administered in clinical trials are 1,800 mg once daily. More frequent ingestion of TYKERB could result in serum concentrations exceeding those observed in clinical trials and could result in increased toxicity. Therefore, missed doses should not be replaced and dosing should resume with the next scheduled daily dose.

There has been a report of one patient who took 3,000 mg of TYKERB for 10 days. This patient had Grade 3 diarrhea and vomiting on Day 10. The event resolved following IV hydration and interruption of treatment with TYKERB and letrozole.

Because lapatinib is not significantly renally excreted and is highly bound to plasma proteins, hemodialysis would not be expected to be an effective method to enhance the elimination of lapatinib.

## 11 DESCRIPTION

Lapatinib is a small molecule and a member of the 4-anilinoquinazoline class of kinase inhibitors. It is present as the monohydrate of the ditosylate salt, with chemical name *N*-(3-chloro-4-{[(3-fluorophenyl)methyl]oxy}phenyl)-6-[5-({[2-(methylsulfonyl)ethyl]amino}methyl)-2-furanyl]-4-quinazolinamine bis(4-methylbenzenesulfonate) monohydrate. It has the molecular formula $C_{29}H_{26}ClFN_4O_4S$ $(C_7H_8O_3S)_2$ $H_2O$ and a molecular weight of 943.5. Lapatinib ditosylate monohydrate has the following chemical structure:




Lapatinib is a yellow solid, and its solubility in water is 0.007 mg/mL and in 0.1N HCl is 0.001 mg/mL at 25°C.



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Exhibit 1029-63

Each 250 mg tablet of TYKERB contains 405 mg of lapatinib ditosylate monohydrate, equivalent to 398 mg of lapatinib ditosylate or 250 mg lapatinib free base.

The inactive ingredients of TYKERB are: **Tablet Core:** Magnesium stearate, microcrystalline cellulose, povidone, sodium starch glycolate. **Coating:** Orange film-coat: FD&C yellow No. 6/sunset yellow FCF aluminum lake, hypromellose, macrogol/PEG 400, polysorbate 80, titanium dioxide.

## 12 CLINICAL PHARMACOLOGY

### 12.1 Mechanism of Action

Lapatinib is a 4-anilinoquinazoline kinase inhibitor of the intracellular tyrosine kinase domains of both Epidermal Growth Factor Receptor (EGFR [ErbB1]) and of Human Epidermal Receptor Type 2 (HER2 [ErbB2]) receptors (estimated $K_i^{app}$ values of 3nM and 13nM, respectively) with a dissociation half-life of ≥300 minutes. Lapatinib inhibits ErbB-driven tumor cell growth in vitro and in various animal models.

An additive effect was demonstrated in an in vitro study when lapatinib and 5-FU (the active metabolite of capecitabine) were used in combination in the 4 tumor cell lines tested. The growth inhibitory effects of lapatinib were evaluated in trastuzumab-conditioned cell lines. Lapatinib retained significant activity against breast cancer cell lines selected for long-term growth in trastuzumab-containing medium in vitro. These in vitro findings suggest non-cross-resistance between these two agents.

Hormone receptor positive breast cancer cells (with ER [Estrogen Receptor] and/or PgR [Progesterone Receptor]) that coexpress the HER2 tend to be resistant to established endocrine therapies. Similarly, hormone receptor positive breast cancer cells that initially lack EGFR or HER2 upregulate these receptor proteins as the tumor becomes resistant to endocrine therapy.

### 12.3 Pharmacokinetics

Absorption: Absorption following oral administration of TYKERB is incomplete and variable. Serum concentrations appear after a median lag time of 0.25 hours (range 0 to 1.5 hour). Peak plasma concentrations ($C_{max}$) of lapatinib are achieved approximately 4 hours after administration. Daily dosing of TYKERB results in achievement of steady state within 6 to 7 days, indicating an effective half-life of 24 hours.

At the dose of 1,250 mg daily, steady state geometric mean (95% confidence interval) values of $C_{max}$ were 2.43 mcg/mL (1.57 to 3.77 mcg/mL) and AUC were 36.2 mcg.hr/mL (23.4 to 56 mcg.hr/mL).

Divided daily doses of TYKERB resulted in approximately 2-fold higher exposure at steady state (steady state AUC) compared to the same total dose administered once daily.

Systemic exposure to lapatinib is increased when administered with food. Lapatinib AUC values were approximately 3- and 4-fold higher ($C_{max}$ approximately 2.5- and 3-fold higher) when administered with a low fat (5% fat-500 calories) or with a high fat (50% fat-1,000 calories) meal, respectively.

Distribution: Lapatinib is highly bound (>99%) to albumin and alpha-1 acid



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Exhibit 1029-64

glycoprotein. In vitro studies indicate that lapatinib is a substrate for the transporters breast cancer resistance protein (BCRP, ABCG2) and P-glycoprotein (P-gp, ABCB1). Lapatinib has also been shown in vitro to inhibit these efflux transporters, as well as the hepatic uptake transporter OATP 1B1, at clinically relevant concentrations.

Metabolism: Lapatinib undergoes extensive metabolism, primarily by CYP3A4 and CYP3A5, with minor contributions from CYP2C19 and CYP2C8 to a variety of oxidated metabolites, none of which accounts for more than 14% of the dose recovered in the feces or 10% of lapatinib concentration in plasma.

Elimination: At clinical doses, the terminal phase half-life following a single dose was 14.2 hours; accumulation with repeated dosing indicates an effective half-life of 24 hours.

Elimination of lapatinib is predominantly through metabolism by CYP3A4/5 with negligible (<2%) renal excretion. Recovery of parent lapatinib in feces accounts for a median of 27% (range 3 to 67%) of an oral dose.

Effects of Age, Gender, or Race: Studies of the effects of age, gender, or race on the pharmacokinetics of lapatinib have not been performed.

### 12.4 QT Prolongation

The QT prolongation potential of lapatinib was assessed as part of an uncontrolled, open-label dose escalation study in advanced cancer patients. Eighty-one patients received daily doses of lapatinib ranging from 175 mg/day to 1,800 mg/day. Serial ECGs were collected on Day 1 and Day 14 to evaluate the effect of lapatinib on QT intervals. Analysis of the data suggested a consistent concentration-dependent increase in QTc interval.

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Two-year carcinogenicity studies with lapatinib are ongoing.

Lapatinib was not clastogenic or mutagenic in the Chinese hamster ovary chromosome aberration assay, microbial mutagenesis (Ames) assay, human lymphocyte chromosome aberration assay or the in vivo rat bone marrow chromosome aberration assay at single doses up to 2,000 mg/kg. However, an impurity in the drug product (up to 4 ppm or 8 mcg/day) was genotoxic when tested alone in both in vitro and in vivo assays.

There were no effects on male or female rat mating or fertility at doses up to 120 mg/kg/day in females and 180 mg/kg/day in males (approximately 6.4 times and 2.6 times the expected human clinical exposure based on AUC following 1,250 mg dose of lapatinib plus capecitabine, respectively). The effect of lapatinib on human fertility is unknown. However, when female rats were given oral doses of lapatinib during breeding and through the first 6 days of gestation, a significant decrease in the number of live fetuses was seen at 120 mg/kg/day and in the fetal body weights at ≥60 mg/kg/day (approximately 6.4 times and 3.3 times the expected human clinical exposure based on AUC following 1,250 mg dose of lapatinib plus capecitabine, respectively).



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Exhibit 1029-65

## 14 CLINICAL STUDIES

### 14.1 HER2 Positive Metastatic Breast Cancer

The efficacy and safety of TYKERB in combination with capecitabine in breast cancer were evaluated in a randomized, Phase 3 trial. Patients eligible for enrollment had HER2 (ErbB2) overexpressing (IHC 3+ or IHC 2+ confirmed by FISH), locally advanced or metastatic breast cancer, progressing after prior treatment that included anthracyclines, taxanes, and trastuzumab.

Patients were randomized to receive either TYKERB 1,250 mg once daily (continuously) plus capecitabine 2,000 $mg/m^2$/day on Days 1-14 every 21 days, or to receive capecitabine alone at a dose of 2,500 $mg/m^2$/day on Days 1-14 every 21 days. The endpoint was time to progression (TTP). TTP was defined as time from randomization to tumor progression or death related to breast cancer. Based on the results of a pre-specified interim analysis, further enrollment was discontinued. Three hundred and ninety-nine (399) patients were enrolled in this study. The median age was 53 years and 14% were older than 65 years. Ninety-one percent (91%) were Caucasian. Ninety-seven percent (97%) had stage IV breast cancer, 48% were estrogen receptor+ (ER+) or progesterone receptor+ (PR+), and 95% were ErbB2 IHC 3+ or IHC 2+ with FISH confirmation. Approximately 95% of patients had prior treatment with anthracyclines, taxanes, and trastuzumab.

Efficacy analyses 4 months after the interim analysis are presented in Table 5, Figure 1, and Figure 2.

**Table 5. Efficacy Results**

| | **Independent Assessment[a]** | | **Investigator Assessment** | |
|---|---|---|---|---|
| | **TYKERB 1,250 mg/day + Capecitabine 2,000 $mg/m^2$/day (N = 198)** | **Capecitabine 2,500 $mg/m^2$/day (N = 201)** | **TYKERB 1,250 mg/day + Capecitabine 2,000 $mg/m^2$/day (N = 198)** | **Capecitabine 2,500 $mg/m^2$/day (N = 201)** |
| **Number of TTP events** | 82 | 102 | 121 | 126 |
| **Median TTP, weeks** | 27.1 | 18.6 | 23.9 | 18.3 |
| ($25^{th}$, $75^{th}$, Percentile), weeks | (17.4, 49.4) | (9.1, 36.9) | (12.0, 44.0) | (6.9, 35.7) |
| **Hazard Ratio** | 0.57 | | 0.72 | |
| (95% CI) | (0.43, 0.77) | | (0.56, 0.92) | |
| *P* value | 0.00013 | | 0.00762 | |
| **Response Rate (%)** | 23.7 | 13.9 | 31.8 | 17.4 |
| (95% CI) | (18.0, 30.3) | (9.5, 19.5) | (25.4, 38.8) | (12.4, 23.4) |

TTP = Time to progression.

[a] The time from last tumor assessment to the data cut-off date was >100 days in approximately 30% of patients in the independent assessment. The pre-specified assessment interval was 42 or 84 days.



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Exhibit 1029-66

**Figure 1. Kaplan-Meier Estimates for Independent Review Panel-evaluated Time to Progression**

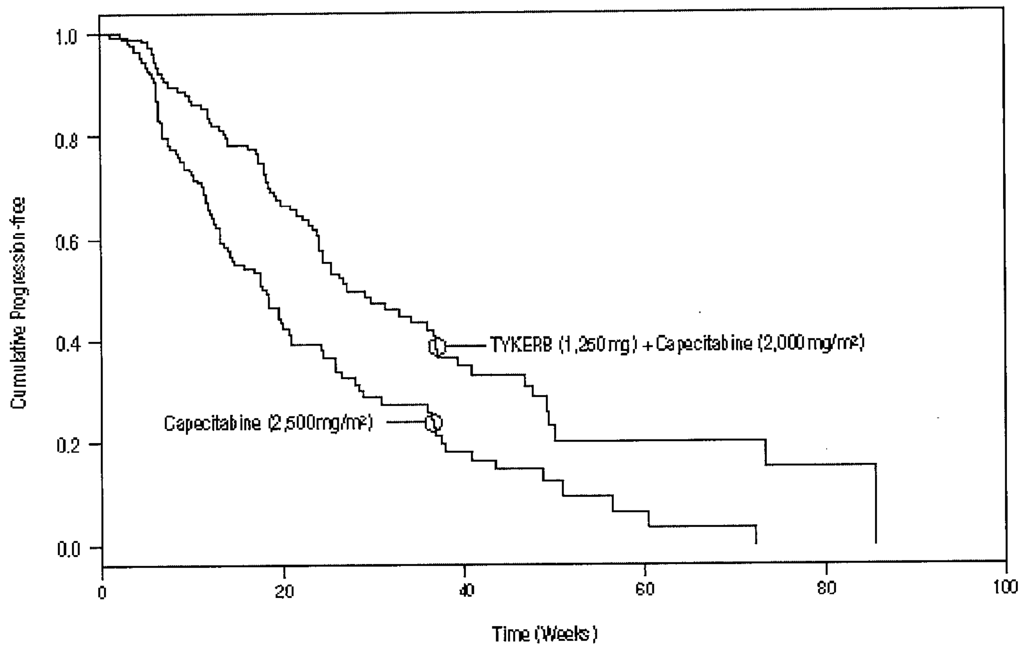


**Figure 2. Kaplan-Meier Estimates for Investigator Assessment Time to Progression**

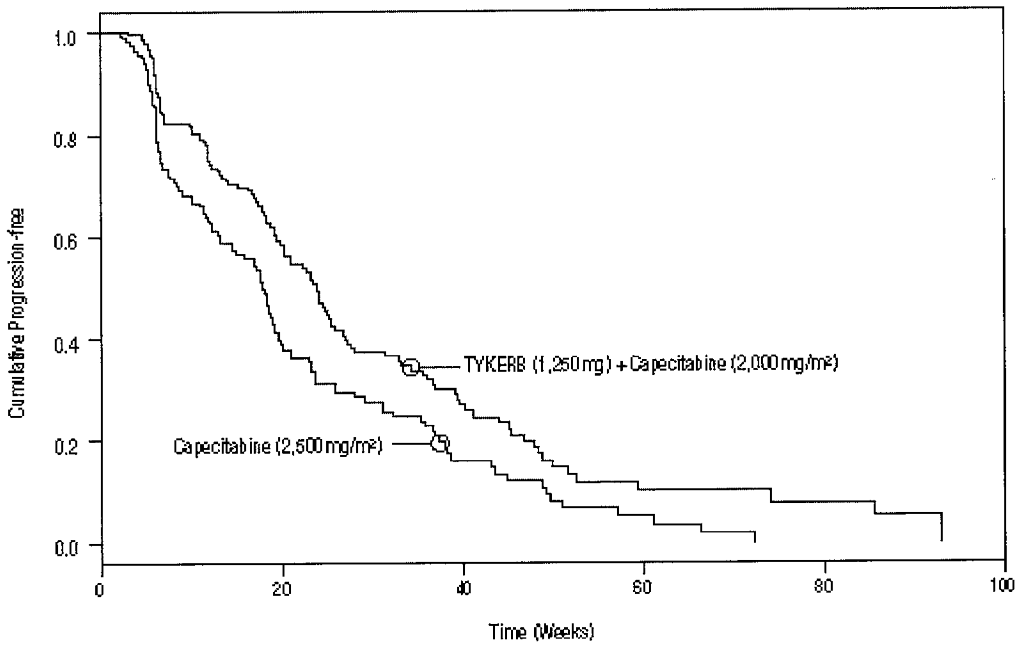




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Exhibit 1029-67

At the time of above efficacy analysis, the overall survival data were not mature (32% events). However, based on the TTP results, the study was unblinded and patients receiving capecitabine alone were allowed to cross over to TYKERB plus capecitabine treatment. The survival data were followed for an additional 2 years to be mature and the analysis is summarized in Table 6.

**Table 6: Overall Survival Data**

| | **TYKERB 1,250 mg/day + Capecitabine 2,000 mg/m²/day (N = 207)** | **Capecitabine 2,500 mg/m²/day (N = 201)** |
|---|---|---|
| **Overall Survival** | | |
| Died | 76% | 82% |
| Median Overall Survival (weeks) | 75.0 | 65.9 |
| Hazard ratio, 95% CI (*P* value) | 0.89 (0.71, 1.10) 0.276 | |

CI = confidence interval

## 14.2 Hormone Receptor Positive, HER2 Positive Metastatic Breast Cancer

The efficacy and safety of TYKERB in combination with letrozole were evaluated in a double-blind, placebo-controlled, multi-center study. A total of 1,286 postmenopausal women with hormone receptor positive (ER positive and/or PgR positive) metastatic breast cancer, who had not received prior therapy for metastatic disease, were randomly assigned to receive either TYKERB (1,500 mg once daily) plus letrozole (2.5 mg once daily) (n = 642) or letrozole (2.5 mg once daily) alone (n = 644). Of all patients randomized to treatment, 219 (17%) patients had tumors overexpressing the HER2 receptor, defined as fluorescence in situ hybridization (FISH) ≥2 or 3+ immunohistochemistry (IHC). There were 952 (74%) patients who were HER2 negative and 115 (9%) patients did not have their HER2 receptor status confirmed. The primary objective was to evaluate and compare progression-free survival (PFS) in the HER2 positive population. Progression-free survival was defined as the interval of time between date of randomization and the earlier date of first documented sign of disease progression or death due to any cause.

The baseline demographic and disease characteristics were balanced between the two treatment arms. The median age was 63 years and 45% were 65 years of age or older. Eighty-four percent (84%) of the patients were White. Approximately 50% of the HER2 positive population had prior adjuvant/neo-adjuvant chemotherapy and 56% had prior hormonal therapy. Only 2 patients had prior trastuzumab.

In the HER2 positive subgroup (n = 219), the addition of TYKERB to letrozole resulted in an improvement in PFS. In the HER2 negative subgroup, there was no improvement in PFS of the TYKERB plus letrozole combination compared to the letrozole plus placebo. Overall



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Exhibit 1029-68

response rate (ORR) was also improved with the TYKERB plus letrozole combination therapy. The overall survival (OS) data were not mature. Efficacy analyses for the hormone receptor positive, HER2 positive and HER2 negative subgroups are presented in Table 7 and Figure 3.

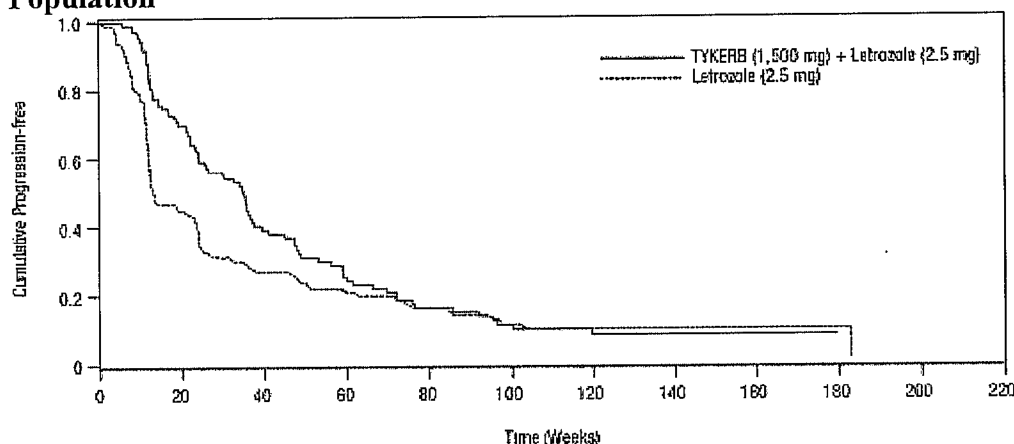
**Table 7. Efficacy Results**

| | **HER2(+) Population** | | **HER2(-) Population** | |
|---|---|---|---|---|
| | **TYKERB 1500 mg/day + Letrozole 2.5 mg/day** | **Letrozole 2.5 mg/day** | **TYKERB 1500 mg/day + Letrozole 2.5 mg/day** | **Letrozole 2.5 mg/day** |
| | **(N = 111)** | **(N = 108)** | **(N = 478)** | **(N = 474)** |
| **Median PFS[a], weeks (95% CI)** | 35.4 (24.1, 39.4) | 13.0 (12.0, 23.7) | 59.7 (48.6, 69.7) | 58.3 (47.9, 62.0) |
| **Hazard Ratio (95% CI)** | 0.71 (0.53, 0.96) | | 0.90 (0.77, 1.05) | |
| ***P* value** | 0.019 | | 0.188 | |
| **Response Rate (%) (95% CI)** | 27.9 (19.8, 37.2) | 14.8 (8.7, 22.9) | 32.6 (28.4, 37.0) | 31.6 (27.5, 36.0) |

PFS = progression-free survival; CI = confidence interval.

[a] Kaplan-Meier estimate.

**Figure 3. Kaplan-Meier Estimates for Progression-Free Survival for the HER2 Positive Population**




## 16 HOW SUPPLIED/STORAGE AND HANDLING

The 250 mg tablets of TYKERB are oval, biconvex, orange, and film-coated with GS XJG debossed on one side and are available in:

Bottles of 150 tablets: NDC 0173-0752-00

Store at 25°C (77°F); excursions permitted to 15° to 30°C (59° to 86°F) [see USP Controlled Room Temperature].



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Exhibit 1029-69

## 17 PATIENT COUNSELING INFORMATION

*See FDA-approved patient labeling (17.2).*

### 17.1 Information for Patients

Patients should be informed of the following:

- TYKERB has been reported to decrease left ventricular ejection fraction which may result in shortness of breath, palpitations, and/or fatigue. Patients should inform their physician if they develop these symptoms while taking TYKERB.
- TYKERB often causes diarrhea which may be severe in some cases. Patients should be told how to manage and/or prevent diarrhea and to inform their physician if severe diarrhea occurs during treatment with TYKERB.
- TYKERB may interact with many drugs; therefore, patients should be advised to report to their healthcare provider the use of any other prescription or nonprescription medication or herbal products.
- TYKERB may interact with grapefruit. Patients should not take TYKERB with grapefruit products.
- TYKERB should be taken at least one hour before or one hour after a meal, in contrast to capecitabine which should be taken with food or within 30 minutes after food.
- The dose of TYKERB should be taken once daily. Dividing the daily dose is not recommended.

### 17.2 FDA-Approved Patient Labeling

Patient labeling is provided as a tear-off leaflet at the end of this full prescribing information.

TYKERB is a registered trademark of GlaxoSmithKline.

GlaxoSmithKline
Research Triangle Park, NC 27709

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January 2010
TKB:6PI



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Exhibit 1029-70

PHARMACIST - DETACH HERE AND GIVE INSTRUCTIONS TO PATIENT

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# PATIENT INFORMATION

## TYKERB (TIE-curb)
## (lapatinib) tablets

Read this leaflet before you start taking TYKERB® and each time you get a refill. There may be new information. This information does not take the place of talking with your doctor about your medical condition or treatment.

**What is TYKERB?**

TYKERB is used with the medicine capecitabine for the treatment of patients with advanced or metastatic breast cancer that is HER2 positive (tumors that produce large amounts of a protein called human epidermal growth factor receptor-2), and who have already had certain other breast cancer treatments.

TYKERB is also used with a type of medicine called letrozole for the treatment of postmenopausal women with hormone receptor positive, HER2 positive metastatic breast cancer for whom hormonal therapy is indicated. TYKERB in combination with an aromatase inhibitor has not been compared to a trastuzumab-containing chemotherapy regimen for the treatment of metastatic breast cancer.

**Who should not take TYKERB?**

Do not take TYKERB if you are allergic to any of its ingredients. See the end of this leaflet for a list of ingredients in TYKERB.

**Before you start taking TYKERB**, tell your doctor about all of your medical conditions, including if you:

- ever had a severe allergic (hypersensitivity) reaction to TYKERB. Check with your doctor if you think this applies to you. Don't take TYKERB.
- have heart problems.
- have liver problems. You may need a lower dose of TYKERB.
- are pregnant or may become pregnant. TYKERB may harm an unborn baby. If you become pregnant during treatment with TYKERB, tell your doctor as soon as possible.
- are breast-feeding. It is not known if TYKERB passes into your breast milk or if it can harm your baby. If you are a woman who has or will have a baby, talk with your doctor about the best way to feed your baby.



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Exhibit 1029-71

Tell your doctor about all the medicines you take, including prescription and nonprescription medicines, vitamins, and herbal and dietary supplements. TYKERB and many other medicines may interact with each other. Your doctor needs to know what medicines you take so he or she can choose the right dose of TYKERB for you.

Especially tell your doctor if you take:

- antibiotics and anti-fungals (drugs used to treat infections)
- HIV (AIDS) treatments
- anticonvulsant drugs (drugs used to treat seizures)
- calcium channel blockers (drugs used to treat certain heart disorders or high blood pressure)
- antidepressants
- drugs used for stomach ulcers
- St. John's Wort or other herbal supplements

Know the medicines you take. Keep a list of your medicines with you to show your doctor. Do not take other medicines during treatment with TYKERB without first checking with your doctor.

Because TYKERB is given with other drugs called capecitabine or letrozole, you should also discuss with your doctor or pharmacist any medicines that should be avoided during treatment.

**How should I take TYKERB?**

- Take TYKERB exactly as your doctor tells you to take it. Your doctor may change your dose of TYKERB if needed.
    - For patients with advanced or metastatic breast cancer, TYKERB and capecitabine are taken in 21 day cycles. The usual dose of TYKERB is 1,250 mg (5 tablets) taken by mouth all at once, **one time a day on days 1 to 21**. Your doctor will tell you the dose of capecitabine you should take and when you should take it.
    - For patients with hormone receptor positive, HER2 positive breast cancer, TYKERB and letrozole are taken daily. The usual dose of TYKERB is 1,500 mg (6 tablets) taken by mouth all at once, **one time a day**. Your doctor will tell you the dose of letrozole you should take and when you should take it.
- TYKERB should be taken at least one hour before, or at least one hour after food.
- Do not eat or drink grapefruit products while taking TYKERB.
- If you forget to take your dose of TYKERB, do not take two doses at one time. Take your next dose at your scheduled time.

**What are the possible side effects of TYKERB?**
**Serious side effects** include:



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Exhibit 1029-72

- **heart problems** including, decreased pumping of blood from the heart and an abnormal heartbeat. Signs and symptoms of an abnormal heartbeat include:
  - feeling like your heart is pounding or racing
  - dizziness
  - tiredness
  - feeling lightheaded
  - shortness of breath
    - Your doctor should check your heart function before you start taking TYKERB and during treatment.
- **liver problems.** Signs and symptoms of liver problems include:
  - itching
  - yellow eyes or skin
  - dark urine
  - pain or discomfort in the right upper stomach area
  - death
    - Your doctor should do blood tests to check your liver before you start taking TYKERB and during treatment.
- **diarrhea**, which may cause you to become dehydrated. Follow your doctors instructions for what to do to help prevent or treat diarrhea.
- **lung problems.** Symptoms of a lung problem with TYKERB include a cough that will not go away or shortness of breath.

**Call your doctor right away if you have any of the signs or symptoms of the serious side effects listed above.**

**Common side effects** of TYKERB in combination with capecitabine or letrozole include:

- diarrhea
- red, painful hands and feet
- nausea
- rash
- vomiting
- tiredness or weakness
- mouth sores
- loss of appetite
- indigestion
- unusual hair loss or thinning
- nose bleeds
- headache
- dry skin
- itching



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- nail disorders such as nail bed changes, nail pain, infection and swelling of the cuticles.

Tell your doctor about any side effect that gets serious or that does not go away.

These are not all the side effects with TYKERB. Ask your doctor or pharmacist for more information.

Call your doctor for medical advice about side effects. You may report side effects to FDA at 1-800-FDA-1088.

**You may also get side effects from the other drugs taken with TYKERB.** Talk to your doctor about possible side effects you may get during treatment.

**How should I store TYKERB tablets?**

- Store TYKERB tablets at room temperature at 59° to 86°F (15° to 30°C). Keep the container closed tightly.
- Do not keep medicine that is out of date or that you no longer need. Be sure that if you throw any medicine away, it is out of the reach of children.
- **Keep TYKERB and all medicines out of the reach of children.**

**General information about TYKERB**

Medicines are sometimes prescribed for conditions that are not mentioned in patient information leaflets. Do not use TYKERB for any other condition for which it was not prescribed. Do not give TYKERB to other people, even if they have the same condition that you have. It may harm them.

This leaflet summarizes the most important information about TYKERB. If you would like more information, talk with your doctor. You can ask your doctor or pharmacist for information about TYKERB that is written for health professionals. For more information, you can call toll-free 1-888-825-5249 or by visiting the website www.tykerb.com.

**What are the ingredients in TYKERB?**

**Active Ingredient:** Lapatinib.

**Inactive Ingredients: Tablet Core:** Magnesium stearate, microcrystalline cellulose, povidone, sodium starch glycolate. **Coating:** Orange film-coat: FD&C yellow #6/sunset yellow FCF aluminum lake, hypromellose, macrogol/PEG 400, polysorbate 80, titanium dioxide.

TYKERB tablets are oval, biconvex, orange, film-coated with GS XJG printed on one side.

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TYKERB is a registered trademark of GlaxoSmithKline.

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Research Triangle Park, NC 27709

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TKB:5PIL



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# EXHIBIT H

HIGHLIGHTS OF PRESCRIBING INFORMATION

**These highlights do not include all the information needed to use IXEMPRA® safely and effectively. See full prescribing information for IXEMPRA®.**

**IXEMPRA® *Kit* (ixabepilone) for Injection, for intravenous infusion only**
**Initial U.S. Approval: 2007**

**WARNING: TOXICITY IN HEPATIC IMPAIRMENT**
***See full prescribing information for complete boxed warning.***
**IXEMPRA® in combination with capecitabine must not be given to patients with AST or ALT >2.5 x ULN or bilirubin >1 x ULN due to increased risk of toxicity and neutropenia-related death. (4, 5.3)**

**RECENT MAJOR CHANGES**

Dosage and Administration, Instructions for Preparation and IV Administration (2.4) 03/2009

**INDICATIONS AND USAGE**

- IXEMPRA, a microtubule inhibitor, in combination with capecitabine is indicated for the treatment of metastatic or locally advanced breast cancer in patients after failure of an anthracycline and a taxane (1).
- IXEMPRA as monotherapy is indicated for the treatment of metastatic or locally advanced breast cancer in patients after failure of an anthracycline, a taxane, and capecitabine (1).

**DOSAGE AND ADMINISTRATION**

- The recommended dose of IXEMPRA is 40 mg/m$^2$ infused intravenously over 3 hours every 3 weeks (2.1).
- Dose reduction is required in certain patients with elevated AST, ALT, or bilirubin (2.2, 8.6).

IXEMPRA (ixabepilone) for injection must be constituted with supplied DILUENT. The ixabepilone concentration in constituted solution is 2 mg/mL.
Constituted solution must be diluted with one of the specified fluids, to a final ixabepilone concentration of 0.2 mg/mL to 0.6 mg/mL. The final solution must be used within 6 hours of preparation (2.4).

**DOSAGE FORMS AND STRENGTHS**

- IXEMPRA for injection, 15 mg supplied with DILUENT for IXEMPRA, 8 mL (3)
- IXEMPRA for injection, 45 mg supplied with DILUENT for IXEMPRA, 23.5 mL (3)

**CONTRAINDICATIONS**

- Hypersensitivity to drugs formulated with Cremophor® EL (4).
- Baseline neutrophil count <1500 cells/mm$^3$ or a platelet count <100,000 cells/mm$^3$ (4).
- Patients with AST or ALT >2.5 x ULN or bilirubin >1 x ULN must not be treated with IXEMPRA (ixabepilone) in combination with capecitabine (4).

**WARNINGS AND PRECAUTIONS**

- Peripheral Neuropathy: Monitor for symptoms of neuropathy, primarily sensory. Neuropathy is cumulative, generally reversible and should be managed by dose adjustment and delays (2.2, 5.1).
- Myelosuppression: Primarily neutropenia. Monitor with peripheral blood cell counts and adjust dose as appropriate (2.2, 5.2).
- Hypersensitivity reaction: Must premedicate all patients with an $H_1$ antagonist and an $H_2$ antagonist before treatment (2.3, 5.4).
- Fetal harm can occur when administered to a pregnant woman. Women should be advised not to become pregnant when taking IXEMPRA (5.5, 8.1).

**ADVERSE REACTIONS**

- The most common adverse reactions (≥20%) are peripheral sensory neuropathy, fatigue/asthenia, myalgia/arthralgia, alopecia, nausea, vomiting, stomatitis/mucositis, diarrhea, and musculoskeletal pain. Additional reactions occurred in ≥20% in combination treatment: palmar-plantar erythrodysesthesia syndrome, anorexia, abdominal pain, nail disorder, and constipation (6).
- Drug-associated hematologic abnormalities (>40%) include neutropenia, leukopenia, anemia, and thrombocytopenia (6).

**To report SUSPECTED ADVERSE REACTIONS, contact Bristol-Myers Squibb at 1-800-721-5072 or FDA at 1-800-FDA-1088 or www.fda.gov/medwatch**

**DRUG INTERACTIONS**

- Inhibitors of CYP3A4 may increase plasma concentrations of ixabepilone; dose of IXEMPRA must be reduced with strong CYP3A4 inhibitors (7.1).
- Inducers of CYP3A4 may decrease plasma concentrations of ixabepilone; alternative therapeutic agents with low enzyme induction potential should be considered (7.1).

**See 17 for PATIENT COUNSELING INFORMATION and FDA-Approved Patient Labeling**

**Revised: 10/2009**

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**WARNING: TOXICITY IN HEPATIC IMPAIRMENT**
**IXEMPRA (ixabepilone) in combination with capecitabine is contraindicated in patients with AST or ALT >2.5 x ULN or bilirubin >1 x ULN due to increased risk of toxicity and neutropenia-related death [*see Contraindications (4) and Warnings and Precautions (5.3)*].**

## 1 INDICATIONS AND USAGE

IXEMPRA (ixabepilone) is indicated in combination with capecitabine for the treatment of patients with metastatic or locally advanced breast cancer resistant to treatment with an anthracycline and a taxane, or whose cancer is taxane resistant and for whom further anthracycline therapy is contraindicated. Anthracycline resistance is defined as progression while on therapy or within 6 months in the adjuvant setting or 3 months in the metastatic setting. Taxane resistance is defined as progression while on therapy or within 12 months in the adjuvant setting or 4 months in the metastatic setting.

IXEMPRA is indicated as monotherapy for the treatment of metastatic or locally advanced breast cancer in patients whose tumors are resistant or refractory to anthracyclines, taxanes, and capecitabine.

## 2 DOSAGE AND ADMINISTRATION

### 2.1 General Dosing Information

The recommended dosage of IXEMPRA is 40 mg/m$^2$ administered intravenously over 3 hours every 3 weeks. Doses for patients with body surface area (BSA) greater than 2.2 m$^2$ should be calculated based on 2.2 m$^2$.

### 2.2 Dose Modification

**Dose Adjustments During Treatment**

Patients should be evaluated during treatment by periodic clinical observation and laboratory tests including complete blood cell counts. If toxicities are present, treatment should be delayed to allow recovery. Dosing adjustment guidelines for monotherapy and combination therapy are shown in Table 1. If toxicities recur, an additional 20% dose reduction should be made.

**Table 1: Dose Adjustment Guidelines[a]**

| IXEMPRA (Monotherapy or Combination Therapy) | IXEMPRA Dose Modification |
|---|---|
| **Nonhematologic:** | |
| Grade 2 neuropathy (moderate) lasting ≥7 days | Decrease the dose by 20% |
| Grade 3 neuropathy (severe) lasting <7 days | Decrease the dose by 20% |
| Grade 3 neuropathy (severe) lasting ≥7 days or disabling neuropathy | Discontinue treatment |
| Any grade 3 toxicity (severe) other than neuropathy | Decrease the dose by 20% |
| Transient grade 3 arthralgia/myalgia or fatigue<br>Grade 3 hand-foot syndrome (palmar-plantar erythrodysesthesia) | No change in dose of IXEMPRA |
| Any grade 4 toxicity (disabling) | Discontinue treatment |
| **Hematologic:** | |
| Neutrophil <500 cells/mm$^3$ for ≥7 days | Decrease the dose by 20% |
| Febrile neutropenia | Decrease the dose by 20% |
| Platelets <25,000/mm$^3$ or platelets <50,000/mm$^3$ with bleeding | Decrease the dose by 20% |
| **CAPECITABINE (when used in combination with IXEMPRA)** | **Capecitabine Dose Modification** |
| **Nonhematologic:** | Follow Capecitabine Label |
| **Hematologic:** | |
| Platelets <25,000/mm$^3$ or <50,000/mm$^3$ with bleeding | Hold for concurrent diarrhea or stomatitis until platelet count >50,000/mm$^3$, then continue at same dose. |
| Neutrophils <500 cells/mm$^3$ for ≥7 days or febrile neutropenia | Hold for concurrent diarrhea or stomatitis until neutrophil count >1,000 cells/mm$^3$, then continue at same dose. |

[a] Toxicities graded in accordance with National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE v3.0).

***Re-treatment Criteria:*** Dose adjustments at the start of a cycle should be based on nonhematologic toxicity or blood counts from the preceding cycle following the guidelines in Table 1. Patients should not begin a new cycle of treatment unless the neutrophil count is at least 1500 cells/mm$^3$, the platelet count is at least 100,000 cells/mm$^3$, and nonhematologic toxicities have improved to grade 1 (mild) or resolved.

**Dose Adjustments in Special Populations - Hepatic Impairment**

***Combination Therapy:***

IXEMPRA in combination with capecitabine is contraindicated in patients with AST or ALT >2.5 x ULN or bilirubin >1 x ULN. Patients receiving combination treatment who have AST and ALT ≤2.5 x ULN and bilirubin ≤1 x ULN may receive the standard dose of ixabepilone (40 mg/m$^2$) [*see Boxed Warning, Contraindications (4), Warnings and Precautions (5.3), and Use in Specific Populations (8.6)*].

***Monotherapy:***

Patients with hepatic impairment should be dosed with IXEMPRA based on the guidelines in Table 2. Patients with moderate hepatic impairment should be started at 20 mg/m$^2$, the dosage in subsequent cycles may be escalated up to, but not exceeding, 30 mg/m$^2$ if tolerated. Use in patients with AST or ALT >10 x ULN or bilirubin >3 x ULN is not recommended. Limited data are available for patients with baseline AST or ALT >5 x ULN. Caution should be used when treating these patients [*see Warnings and Precautions (5.3) and Use in Specific Populations (8.6)*].

**Table 2: Dose Adjustments for IXEMPRA as Monotherapy in Patients with Hepatic Impairment**

| | Transaminase Levels | | Bilirubin Levels[a] | IXEMPRA[b] (mg/m$^2$) |
|---|---|---|---|---|
| Mild | AST and ALT ≤2.5 x ULN | and | ≤1 x ULN | 40 |
| | AST and ALT ≤10 x ULN | and | ≤1.5 x ULN | 32 |
| Moderate | AST and ALT ≤10 x ULN | and | >1.5 x ULN - ≤3 x ULN | 20 - 30 |

[a] Excluding patients whose total bilirubin is elevated due to Gilbert's disease.

[b] Dosage recommendations are for first course of therapy; further decreases in subsequent courses should be based on individual tolerance.

**Strong CYP3A4 Inhibitors**

The use of concomitant strong CYP3A4 inhibitors should be avoided (eg, ketoconazole, itraconazole, clarithromycin, atazanavir, nefazodone, saquinavir, telithromycin, ritonavir, amprenavir, indinavir, nelfinavir, delavirdine, or voriconazole). Grapefruit juice may also increase plasma concentrations of IXEMPRA and should be avoided. Based on pharmacokinetic studies, if a strong CYP3A4 inhibitor must be coadministered, a dose reduction to 20 mg/m$^2$ is predicted to adjust the ixabepilone AUC to the range observed without inhibitors and should be considered. If the strong inhibitor is discontinued, a washout period of approximately 1 week should be allowed before the IXEMPRA dose is adjusted upward to the indicated dose [*see Drug Interactions (7.1)*].

### 2.3 Premedication

To minimize the chance of occurrence of a hypersensitivity reaction, all patients must be premedicated approximately 1 hour before the infusion of IXEMPRA with:

- An $H_1$ antagonist (eg, diphenhydramine 50 mg orally or equivalent) and
- An $H_2$ antagonist (eg, ranitidine 150 - 300 mg orally or equivalent).

Patients who experienced a hypersensitivity reaction to IXEMPRA require premedication with corticosteroids (eg, dexamethasone 20 mg intravenously, 30 minutes before infusion or orally, 60 minutes before infusion) in addition to pretreatment with $H_1$ and $H_2$ antagonists.

### 2.4 Instructions for Preparation and IV Administration

IXEMPRA *Kit* contains two vials, a vial labeled IXEMPRA (ixabepilone) for injection which contains ixabepilone powder and a vial containing DILUENT for IXEMPRA. Only supplied DILUENT must be used for constituting IXEMPRA (ixabepilone) for injection. IXEMPRA *Kit* must be stored in a refrigerator at 2° C - 8° C (36° F - 46° F) in the original package to protect from light. Prior to constituting IXEMPRA for injection, the *Kit* should be removed from the refrigerator and allowed to stand at room temperature for approximately 30 minutes. When the vials are first removed from the refrigerator, a white precipitate may be observed in the DILUENT vial. This precipitate will dissolve to form a clear solution once the DILUENT warms to room temperature. To allow for withdrawal losses, the vial labeled as 15 mg IXEMPRA for injection contains 16 mg of ixabepilone and the vial labeled as 45 mg IXEMPRA for injection contains 47 mg of ixabepilone. The 15-mg IXEMPRA *Kit* is supplied with a vial providing 8 mL of the DILUENT and the 45-mg IXEMPRA *Kit* is supplied with a vial providing 23.5 mL of the DILUENT. After constituting with the DILUENT, the concentration of ixabepilone is 2 mg/mL.

***Please refer to Preparation and Handling Precautions [see Dosage and Administration (2.5)] before preparation.***

**A. To constitute:**

1. With a suitable syringe, aseptically withdraw the DILUENT and slowly inject it into the IXEMPRA for injection vial. The 15-mg IXEMPRA is constituted with 8 mL of DILUENT and the 45-mg IXEMPRA is constituted with 23.5 mL of DILUENT.
2. Gently swirl and invert the vial until the powder in IXEMPRA is completely dissolved.

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**B. To dilute:**

Before administration, the constituted solution must be further diluted with one of the specified infusion fluids listed below. The IXEMPRA infusion must be prepared in a DEHP [di-(2-ethylhexyl) phthalate] free bag.

The following infusion fluids have been qualified for use in the dilution of IXEMPRA:

- Lactated Ringer's Injection, USP
- 0.9% Sodium Chloride Injection, USP (pH adjusted with Sodium Bicarbonate Injection, USP)
  - When using a 250 mL or a 500 mL bag of 0.9% Sodium Chloride Injection to prepare the infusion, the pH must be adjusted to a pH between 6.0 and 9.0 by adding 2 mEq (ie, 2 mL of an 8.4% w/v solution or 4 mL of a 4.2% w/v solution) of Sodium Bicarbonate Injection, prior to the addition of the constituted IXEMPRA solution.
- PLASMA-LYTE A Injection pH 7.4®

For most doses, a 250 mL bag of infusion fluid is sufficient. However, it is necessary to check the final IXEMPRA infusion concentration of each dose based on the volume of infusion fluid to be used.

The final concentration for infusion must be between 0.2 mg/mL and 0.6 mg/mL. To calculate the final infusion concentration, use the following formulas:

**Total Infusion Volume = mL of Constituted Solution + mL of infusion fluid**

**Final Infusion Concentration = Dose of IXEMPRA (mg)/Total Infusion Volume (mL)**

1. Aseptically, withdraw the appropriate volume of constituted solution containing 2 mg of ixabepilone per mL.
2. Aseptically, transfer to an intravenous (IV) bag containing an appropriate volume of infusion fluid to achieve the final desired concentration of IXEMPRA.
3. Thoroughly mix the infusion bag by manual rotation.

The infusion solution must be administered through an appropriate in-line filter with a microporous membrane of 0.2 to 1.2 microns. DEHP-free infusion containers and administration sets must be used. Any remaining solution should be discarded according to institutional procedures for antineoplastics.

**Stability**

After constituting IXEMPRA, the constituted solution should be further diluted with infusion fluid as soon as possible, but may be stored in the vial (not the syringe) for a maximum of 1 hour at room temperature and room light. Once diluted with infusion fluid, the solution is stable at room temperature and room light for a maximum of 6 hours. Administration of diluted IXEMPRA must be completed within this 6-hour period. The infusion fluids previously mentioned are specified because their pH is in the range of 6.0 to 9.0, which is required to maintain IXEMPRA stability. Other infusion fluids should not be used with IXEMPRA.

### 2.5 Preparation and Handling Precautions

Procedures for proper handling and disposal of antineoplastic drugs [*see References (15)*] should be followed. To minimize the risk of dermal exposure, impervious gloves should be worn when handling vials containing IXEMPRA, regardless of the setting, including unpacking and inspection, transport within a facility, and dose preparation and administration.

## 3 DOSAGE FORMS AND STRENGTHS

IXEMPRA for injection, 15 mg supplied with DILUENT for IXEMPRA, 8 mL.

IXEMPRA for injection, 45 mg supplied with DILUENT for IXEMPRA, 23.5 mL.

## 4 CONTRAINDICATIONS

IXEMPRA is contraindicated in patients with a history of a severe (CTC grade 3/4) hypersensitivity reaction to agents containing Cremophor® EL or its derivatives (eg, polyoxyethylated castor oil) [*see Warnings and Precautions (5.4)*].

IXEMPRA is contraindicated in patients who have a neutrophil count <1500 cells/mm$^3$ or a platelet count <100,000 cells/mm$^3$ [*see Warnings and Precautions (5.2)*].

IXEMPRA in combination with capecitabine is contraindicated in patients with AST or ALT >2.5 x ULN or bilirubin >1 x ULN [*see Boxed Warning and Warnings and Precautions (5.3)*].

## 5 WARNINGS AND PRECAUTIONS

### 5.1 Peripheral Neuropathy

Peripheral neuropathy was common (see Table 3). Patients treated with IXEMPRA should be monitored for symptoms of neuropathy, such as burning sensation, hyperesthesia, hypoesthesia, paresthesia, discomfort, or neuropathic pain. Neuropathy occurred early during treatment; ~75% of new onset or worsening neuropathy occurred during the first 3 cycles. Patients experiencing new or worsening symptoms may require a reduction or delay in the dose of IXEMPRA [*see Dosage and Administration (2.2)*]. In clinical studies, peripheral neuropathy was managed through dose reductions, dose delays, and treatment discontinuation. Neuropathy was the most frequent cause of treatment discontinuation due to drug toxicity. In Studies 046 and 081, 80% and 87%, respectively, of patients with peripheral neuropathy who received IXEMPRA had improvement or no worsening of their neuropathy following dose reduction. For patients with grade 3/4 neuropathy in Studies 046 and 081, 76% and 79%, respectively, had documented improvement to baseline or grade 1, twelve weeks after onset.

IXEMPRA® (ixabepilone)

**Table 3: Treatment-related Peripheral Neuropathy**

| | IXEMPRA with capecitabine Study 046 | IXEMPRA as monotherapy Study 081 |
|---|---|---|
| Peripheral neuropathy (all grades)[a,b] | 67% | 63% |
| Peripheral neuropathy (grades 3/4)[a,b] | 23% | 14% |
| Discontinuation due to neuropathy | 21% | 6% |
| Median number of cycles to onset of grade 3/4 neuropathy | 4 | 4 |
| Median time to improvement of grade 3/4 neuropathy to baseline or to grade 1 | 6.0 weeks | 4.6 weeks |

[a] Sensory and motor neuropathy combined.
[b] 24% and 27% of patients in 046 and 081, respectively, had preexisting neuropathy (grade 1).

A pooled analysis of 1540 cancer patients treated with IXEMPRA indicated that patients with diabetes mellitus or preexisting peripheral neuropathy may be at increased risk of severe neuropathy. Prior therapy with neurotoxic chemotherapy agents did not predict the development of neuropathy. Patients with moderate to severe neuropathy (grade 2 or greater) were excluded from studies with IXEMPRA. Caution should be used when treating patients with diabetes mellitus or preexisting peripheral neuropathy.

### 5.2 Myelosuppression

Myelosuppression is dose-dependent and primarily manifested as neutropenia. In clinical studies, grade 4 neutropenia (<500 cells/mm$^3$) occurred in 36% of patients treated with IXEMPRA in combination with capecitabine and 23% of patients treated with IXEMPRA monotherapy. Febrile neutropenia and infection with neutropenia were reported in 5% and 6% of patients treated with IXEMPRA in combination with capecitabine, respectively, and 3% and 5% of patients treated with IXEMPRA as monotherapy, respectively. Neutropenia-related death occurred in 1.9% of 414 patients with normal hepatic function or mild hepatic impairment treated with IXEMPRA in combination with capecitabine. The rate of neutropenia-related deaths was higher (29%, 5 out of 17) in patients with AST or ALT >2.5 x ULN or bilirubin >1.5 x ULN [*see Boxed Warning, Contraindications (4), and Warnings and Precautions (5.3)*]. Neutropenia-related death occurred in 0.4% of 240 patients treated with IXEMPRA as monotherapy. No neutropenia-related deaths were reported in 24 patients with AST or ALT >2.5 x ULN or bilirubin >1.5 x ULN treated with IXEMPRA monotherapy. IXEMPRA must not be administered to patients with a neutrophil count <1500 cells/mm$^3$. To monitor for myelosuppression, frequent peripheral blood cell counts are recommended for all patients receiving IXEMPRA. Patients who experience severe neutropenia or thrombocytopenia should have their dose reduced [*see Dosage and Administration (2.2)*].

### 5.3 Hepatic Impairment

Patients with baseline AST or ALT >2.5 x ULN or bilirubin >1.5 x ULN experienced greater toxicity than patients with baseline AST or ALT ≤2.5 x ULN or bilirubin ≤1.5 x ULN when treated with IXEMPRA at 40 mg/m$^2$ in combination with capecitabine or as monotherapy in breast cancer studies. In combination with capecitabine, the overall frequency of grade 3/4 adverse reactions, febrile neutropenia, serious adverse reactions, and toxicity-related deaths was greater [*see Warnings and Precautions (5.2)*]. With monotherapy, grade 4 neutropenia, febrile neutropenia, and serious adverse reactions were more frequent. The safety and pharmacokinetics of IXEMPRA as monotherapy were evaluated in a dose escalation study in 56 patients with varying degrees of hepatic impairment. Exposure was increased in patients with elevated AST or bilirubin [*see Use in Specific Populations (8.6)*].

IXEMPRA in combination with capecitabine is contraindicated in patients with AST or ALT >2.5 x ULN or bilirubin >1 x ULN due to increased risk of toxicity- and neutropenia-related death [*see Boxed Warning, Contraindications (4), and Warnings and Precautions (5.2)*]. Patients who are treated with IXEMPRA as monotherapy should receive a reduced dose depending on the degree of hepatic impairment [*see Dosage and Administration (2.2)*]. Use in patients with AST or ALT >10 x ULN or bilirubin >3 x ULN is not recommended. Limited data are available for patients with AST or ALT >5 x ULN. Caution should be used when treating these patients [*see Dosage and Administration (2.2)*].

### 5.4 Hypersensitivity Reactions

Patients with a history of a severe hypersensitivity reaction to agents containing Cremophor® EL or its derivatives (eg, polyoxyethylated castor oil) should not be treated with IXEMPRA. All patients should be premedicated with an $H_1$ and an $H_2$ antagonist approximately 1 hour before IXEMPRA infusion and be observed for hypersensitivity reactions (eg, flushing, rash, dyspnea, and bronchospasm). In case of severe hypersensitivity reactions, infusion of IXEMPRA should be stopped and aggressive supportive treatment (eg, epinephrine, corticosteroids) started. Of the 1323 patients treated with IXEMPRA in clinical studies, 9 patients (1%) had experienced severe hypersensitivity reactions (including anaphylaxis). Three of the 9 patients were able to be retreated. Patients who experience a hypersensitivity reaction in one cycle of IXEMPRA must be premedicated in subsequent cycles with a corticosteroid in addition to the $H_1$ and $H_2$ antagonists, and extension of the infusion time should be considered [*see Dosage and Administration (2.3) and Contraindications (4)*].

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Exhibit 1029-79

### 5.5 Pregnancy

Pregnancy Category D.

IXEMPRA may cause fetal harm when administered to pregnant women. There are no adequate and well-controlled studies with IXEMPRA in pregnant women. Women should be advised not to become pregnant when taking IXEMPRA. If this drug is used during pregnancy, or if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus.

Ixabepilone was studied for effects on embryo-fetal development in pregnant rats and rabbits given IV doses of 0.02, 0.08, and 0.3 mg/kg/day and 0.01, 0.03, 0.11, and 0.3 mg/kg/day, respectively. There were no teratogenic effects. In rats, an increase in resorptions and post-implantation loss and a decrease in the number of live fetuses and fetal weight was observed at the maternally toxic dose of 0.3 mg/kg/day (approximately one-tenth the human clinical exposure based on AUC). Abnormalities included a reduced ossification of caudal vertebrae, sternebrae, and metacarpals. In rabbits, ixabepilone caused maternal toxicity (death) and embryo-fetal toxicity (resorptions) at 0.3 mg/kg/day (approximately one-tenth the human clinical dose based on body surface area). No fetuses were available at this dose for evaluation.

### 5.6 Cardiac Adverse Reactions

The frequency of cardiac adverse reactions (myocardial ischemia and ventricular dysfunction) was higher in the IXEMPRA in combination with capecitabine (1.9%) than in the capecitabine alone (0.3%) treatment group. Supraventricular arrhythmias were observed in the combination arm (0.5%) and not in the capecitabine alone arm. Caution should be exercised in patients with a history of cardiac disease. Discontinuation of IXEMPRA should be considered in patients who develop cardiac ischemia or impaired cardiac function.

### 5.7 Potential for Cognitive Impairment from Excipients

Since IXEMPRA contains dehydrated alcohol USP, consideration should be given to the possibility of central nervous system and other effects of alcohol [*see Description (11)*].

## 6 ADVERSE REACTIONS

The following adverse reactions are discussed in greater detail in other sections.

- Peripheral neuropathy [*see Warnings and Precautions (5.1)*]
- Myelosuppression [*see Warnings and Precautions (5.2)*]
- Hypersensitivity reactions [*see Warnings and Precautions (5.4)*]

Because clinical trials are conducted under widely varying conditions, the adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in other clinical trials and may not reflect the rates observed in clinical practice.

Unless otherwise specified, assessment of adverse reactions is based on one randomized study (Study 046) and one single-arm study (Study 081). In Study 046, 369 patients with metastatic breast cancer were treated with IXEMPRA 40 mg/m$^2$ administered intravenously over 3 hours every 21 days, combined with capecitabine 1000 mg/m$^2$ twice daily for 2 weeks followed by a 1-week rest period. Patients treated with capecitabine as monotherapy (n=368) in this study received 1250 mg/m$^2$ twice daily for 2 weeks every 21 days. In Study 081, 126 patients with metastatic or locally advanced breast cancer were treated with IXEMPRA 40 mg/m$^2$ administered intravenously over 3 hours every 3 weeks.

The most common adverse reactions (≥20%) reported by patients receiving IXEMPRA were peripheral sensory neuropathy, fatigue/asthenia, myalgia/arthralgia, alopecia, nausea, vomiting, stomatitis/mucositis, diarrhea, and musculoskeletal pain. The following additional reactions occurred in ≥20% in combination treatment: palmar-plantar erythrodysesthesia (hand-foot) syndrome, anorexia, abdominal pain, nail disorder, and constipation. The most common hematologic abnormalities (>40%) include neutropenia, leukopenia, anemia, and thrombocytopenia.

Table 4 presents nonhematologic adverse reactions reported in 5% or more of patients. Hematologic abnormalities are presented separately in Table 5.

**Table 4: Nonhematologic Drug-related Adverse Reactions Occurring in at Least 5% of Patients with Metastatic or Locally Advanced Breast Cancer Treated with IXEMPRA**

| | Study 046 | | | | Study 081 | |
|---|---|---|---|---|---|---|
| | IXEMPRA with capecitabine n=369 | | Capecitabine n=368 | | IXEMPRA monotherapy n=126 | |
| System Organ Class[a]/ Preferred Term | Total (%) | Grade 3/4 (%) | Total (%) | Grade 3/4 (%) | Total (%) | Grade 3/4 (%) |
| ***Infections and Infestations*** | | | | | | |
| Upper respiratory tract infection[b] | 4 | 0 | 3 | 0 | 6 | 0 |
| ***Blood and Lymphatic System Disorders*** | | | | | | |
| Febrile neutropenia | 5 | 4[c] | 1 | 1[d] | 3 | 3[d] |
| ***Immune System Disorders*** | | | | | | |
| Hypersensitivity[b] | 2 | 1[d] | 0 | 0 | 5 | 1[d] |
| ***Metabolism and Nutrition Disorders*** | | | | | | |
| Anorexia[b] | 34 | 3[d] | 15 | 1[d] | 19 | 2[d] |
| Dehydration[b] | 5 | 2 | 2 | <1[d] | 2 | 1[d] |
| ***Psychiatric*** | | | | | | |
| Insomnia[b] | 9 | <1[d] | 2 | 0 | 5 | 0 |
| ***Nervous System Disorders*** | | | | | | |
| Peripheral neuropathy | | | | | | |
| Sensory neuropathy[b,e] | 65 | 21 | 16 | 0 | 62 | 14 |
| Motor neuropathy[b] | 16 | 5[d] | <1 | 0 | 10 | 1[d] |
| Headache | 8 | <1[d] | 3 | 0 | 11 | 0 |
| Taste disorder[b] | 12 | 0 | 4 | 0 | 6 | 0 |
| Dizziness | 8 | 1[d] | 5 | 1[d] | 7 | 0 |
| ***Eye Disorders*** | | | | | | |
| Lacrimation increased | 5 | 0 | 4 | <1[d] | 4 | 0 |
| ***Vascular Disorders*** | | | | | | |
| Hot flush[b] | 5 | 0 | 2 | 0 | 6 | 0 |
| ***Respiratory, Thoracic, and Mediastinal Disorders*** | | | | | | |
| Dyspnea[b] | 7 | 1 | 4 | 1 | 9 | 1[d] |
| Cough[b] | 6 | 0 | 2 | 0 | 2 | 0 |
| ***Gastrointestinal Disorders*** | | | | | | |
| Nausea | 53 | 3[d] | 40 | 2[d] | 42 | 2[d] |
| Vomiting[b] | 39 | 4[d] | 24 | 2 | 29 | 1[d] |
| Stomatitis/mucositis[b] | 31 | 4 | 20 | 3[d] | 29 | 6 |
| Diarrhea[b] | 44 | 6[d] | 39 | 9 | 22 | 1[d] |
| Constipation | 22 | 0 | 6 | <1[d] | 16 | 2[d] |
| Abdominal pain[b] | 24 | 2[d] | 14 | 1[d] | 13 | 2[d] |
| Gastroesophageal reflux disease[b] | 7 | 1[d] | 8 | 0 | 6 | 0 |
| ***Skin and Subcutaneous Tissue Disorders*** | | | | | | |
| Alopecia[b] | 31 | 0 | 3 | 0 | 48 | 0 |
| Skin rash[b] | 17 | 1[d] | 7 | 0 | 9 | 2[d] |
| Nail disorder[b] | 24 | 2[d] | 10 | <1[d] | 9 | 0 |
| Palmar-plantar erythrodysesthesia syndrome[b,f] | 64 | 18[d] | 63 | 17[d] | 8 | 2[d] |
| Pruritus | 5 | 0 | 2 | 0 | 6 | 1[d] |
| Skin exfoliation[b] | 5 | <1[d] | 3 | 0 | 2 | 0 |
| Skin hyperpigmentation[b] | 11 | 0 | 14 | 0 | 2 | 0 |
| ***Musculoskeletal, Connective Tissue, and Bone Disorders*** | | | | | | |
| Myalgia/arthralgia[b] | 39 | 8[d] | 5 | <1[d] | 49 | 8[d] |
| Musculoskeletal pain[b] | 23 | 2[d] | 5 | 0 | 20 | 3[d] |
| ***General Disorders and Administrative Site Conditions*** | | | | | | |
| Fatigue/asthenia[b] | 60 | 16 | 29 | 4 | 56 | 13 |
| Edema[b] | 8 | 0 | 5 | <1[d] | 9 | 1[d] |
| Pyrexia | 10 | 1[d] | 4 | 0 | 8 | 1[d] |
| Pain[b] | 9 | 1[d] | 2 | 0 | 8 | 3[d] |
| Chest pain[b] | 4 | 1[d] | <1 | 0 | 5 | 1[d] |
| ***Investigations*** | | | | | | |
| Weight decreased | 11 | 0 | 3 | 0 | 6 | 0 |

[a] System organ class presented as outlined in Guidelines for Preparing Core Clinical Safety Information on Drugs by the Council for International Organizations of Medical Sciences (CIOMS).

[b] A composite of multiple MedDRA Preferred Terms.

[c] NCI CTC grading for febrile neutropenia ranges from Grade 3 to 5. Three patients (1%) experienced Grade 5 (fatal) febrile neutropenia. Other neutropenia-related deaths (9) occurred in the absence of reported febrile neutropenia [*see Warnings and Precautions (5.2)*].

[d] No grade 4 reports.

[e] Peripheral sensory neuropathy (graded with the NCI CTC scale) was defined as the occurrence of any of the following: areflexia, burning sensation, dysesthesia, hyperesthesia, hypoesthesia, hyporeflexia, neuralgia, neuritis, neuropathy, neuropathy peripheral, neurotoxicity, painful response to normal stimuli, paresthesia, pallanesthesia, peripheral sensory neuropathy, polyneuropathy, polyneuropathy toxic and sensorimotor disorder.

Peripheral motor neuropathy was defined as the occurrence of any of the following: multifocal motor neuropathy, neuromuscular toxicity, peripheral motor neuropathy, and peripheral sensorimotor neuropathy.

[f] Palmar-plantar erythrodysesthesia (hand-foot syndrome) was graded on a 1-3 severity scale in Study 046.

PHIGENIX
Exhibit 1029-80

**Table 5: Hematologic Abnormalities in Patients with Metastatic or Locally Advanced Breast Cancer Treated with IXEMPRA**

| | Study 046 | | | | Study 081 | |
|---|---|---|---|---|---|---|
| | IXEMPRA with capecitabine n=369 | | Capecitabine n=368 | | IXEMPRA monotherapy n=126 | |
| Hematology Parameter | Grade 3 (%) | Grade 4 (%) | Grade 3 (%) | Grade 4 (%) | Grade 3 (%) | Grade 4 (%) |
| Neutropenia[a] | 32 | 36 | 9 | 2 | 31 | 23 |
| Leukopenia (WBC) | 41 | 16 | 5 | 1 | 36 | 13 |
| Anemia (Hgb) | 8 | 2 | 4 | 1 | 6 | 2 |
| Thrombocytopenia | 5 | 3 | 2 | 2 | 5 | 2 |

[a] G-CSF (granulocyte colony stimulating factor) or GM-CSF (granulocyte macrophage stimulating factor) was used in 20% and 17% of patients who received IXEMPRA in Study 046 and Study 081, respectively.

The following serious adverse reactions were also reported in 1323 patients treated with IXEMPRA as monotherapy or in combination with other therapies in Phase 2 and 3 studies.

***Infections and Infestations:*** sepsis, pneumonia, infection, neutropenic infection, urinary tract infection, bacterial infection, enterocolitis, laryngitis, lower respiratory tract infection

***Blood and Lymphatic System Disorders:*** coagulopathy, lymphopenia

***Metabolism and Nutrition Disorders:*** hyponatremia, metabolic acidosis, hypokalemia, hypovolemia

***Nervous System Disorders:*** cognitive disorder, syncope, cerebral hemorrhage, abnormal coordination, lethargy

***Cardiac Disorders:*** myocardial infarction, supraventricular arrhythmia, left ventricular dysfunction, angina pectoris, atrial flutter, cardiomyopathy, myocardial ischemia

***Vascular Disorders:*** hypotension, thrombosis, embolism, hemorrhage, hypovolemic shock, vasculitis

***Respiratory, Thoracic, and Mediastinal Disorders:*** pneumonitis, hypoxia, respiratory failure, acute pulmonary edema, dysphonia, pharyngolaryngeal pain

***Gastrointestinal Disorders:*** ileus, colitis, impaired gastric emptying, esophagitis, dysphagia, gastritis, gastrointestinal hemorrhage

***Hepatobiliary Disorders:*** acute hepatic failure, jaundice

***Skin and Subcutaneous Tissue Disorders:*** erythema multiforme

***Musculoskeletal, Connective Tissue Disorders, and Bone Disorders:*** muscular weakness, muscle spasms, trismus

***Renal and Urinary Disorders:*** nephrolithiasis, renal failure

***General Disorders and Administration Site Conditions:*** chills

***Investigations:*** increased transaminases, increased blood alkaline phosphatase, increased gamma-glutamyltransferase

## 7 DRUG INTERACTIONS

### 7.1 Effect of Other Drugs on Ixabepilone

***Drugs That May Increase Ixabepilone Plasma Concentrations***

**CYP3A4 Inhibitors:** Co-administration of ixabepilone with ketoconazole, a potent CYP3A4 inhibitor, increased ixabepilone AUC by 79% compared to ixabepilone treatment alone. If alternative treatment cannot be administered, a dose adjustment should be considered. The effect of mild or moderate inhibitors (eg, erythromycin, fluconazole, or verapamil) on exposure to ixabepilone has not been studied. Therefore, caution should be used when administering mild or moderate CYP3A4 inhibitors during treatment with IXEMPRA, and alternative therapeutic agents that do not inhibit CYP3A4 should be considered. Patients receiving CYP3A4 inhibitors during treatment with IXEMPRA should be monitored closely for acute toxicities (eg, frequent monitoring of peripheral blood counts between cycles of IXEMPRA) [*see Dosage and Administration (2.2)*].

***Drugs That May Decrease Ixabepilone Plasma Concentrations***

**CYP3A4 Inducers:** IXEMPRA is a CYP3A4 substrate. Strong CYP3A4 inducers (eg, dexamethasone, phenytoin, carbamazepine, rifampin, rifampicin, rifabutin, and phenobarbital) may decrease ixabepilone concentrations leading to subtherapeutic levels. Therefore, therapeutic agents with low enzyme induction potential should be considered for coadministration with IXEMPRA. St. John's Wort may decrease ixabepilone plasma concentrations unpredictably and should be avoided.

### 7.2 Effect of Ixabepilone on Other Drugs

Ixabepilone does not inhibit CYP enzymes at relevant clinical concentrations and is not expected to alter the plasma concentrations of other drugs [*see Clinical Pharmacology (12.3)*].

### 7.3 Capecitabine

In patients with cancer who received ixabepilone (40 mg/m$^2$) in combination with capecitabine (1000 mg/m$^2$), ixabepilone $C_{max}$ decreased by 19%, capecitabine $C_{max}$ decreased by 27%, and 5-fluorouracil AUC increased by 14%, as compared to ixabepilone or capecitabine administered separately. The interaction is not clinically significant given that the combination treatment is supported by efficacy data.

## 8 USE IN SPECIFIC POPULATIONS

### 8.1 Pregnancy

Pregnancy Category D [*see Warnings and Precautions (5.5)*].

### 8.3 Nursing Mothers

It is not known whether ixabepilone is excreted into human milk. Following intravenous administration of radiolabeled ixabepilone to rats on days 7 to 9 postpartum, concentrations of radioactivity in milk were comparable with those in plasma and declined in parallel with the plasma concentrations. Because many drugs are excreted in human milk and because of the potential for serious adverse reactions in nursing infants from ixabepilone, a decision must be made whether to discontinue nursing or to discontinue IXEMPRA taking into account the importance of the drug to the mother.

### 8.4 Pediatric Use

Safety and effectiveness in pediatric patients have not been established.

### 8.5 Geriatric Use

Clinical studies of IXEMPRA did not include sufficient numbers of subjects aged sixty-five and over to determine whether they respond differently from younger subjects.

Forty-five of 431 patients treated with IXEMPRA in combination with capecitabine were ≥65 years of age and 3 patients were ≥75. Overall, the incidence of grade 3/4 adverse reactions were higher in patients ≥65 years of age versus those <65 years of age (82% versus 68%) including grade 3/4 stomatitis (9% versus 1%), diarrhea (9% versus 6%), palmar-plantar erythrodysesthesia syndrome (27% versus 20%), peripheral neuropathy (24% versus 22%), febrile neutropenia (9% versus 3%), fatigue (16% versus 12%), and asthenia (11% versus 6%). Toxicity-related deaths occurred in 2 (4.7%) of 43 patients ≥65 years with normal baseline hepatic function or mild impairment.

Thirty-two of 240 breast cancer patients treated with IXEMPRA as monotherapy were ≥65 years of age and 6 patients were ≥75. No overall differences in safety were observed in these patients compared to those <65 years of age.

### 8.6 Hepatic Impairment

IXEMPRA was evaluated in 56 patients with mild to severe hepatic impairment defined by bilirubin levels and AST levels. Compared to patients with normal hepatic function (n=17), the area under the curve ($AUC_{0\text{-infinity}}$) of ixabepilone increased by:

- 22% in patients with a) bilirubin >1 – 1.5 x ULN **or** b) AST >ULN but bilirubin <1.5 x ULN;
- 30% in patients with bilirubin >1.5 – 3 x ULN and any AST level; and
- 81% in patients with bilirubin >3 x ULN and any AST level.

Doses of 10 and 20 mg/m$^2$ as monotherapy were tolerated in 17 patients with severe hepatic impairment (bilirubin >3 x ULN).

IXEMPRA in combination with capecitabine must not be given to patients with AST or ALT >2.5 x ULN or bilirubin >1 x ULN [*see Boxed Warning, Contraindications (4), and Warnings and Precautions (5.3)*]. Dose reduction is recommended when administering IXEMPRA as monotherapy to patients with hepatic impairment [*see Dosage and Administration (2.3)*]. Because there is a need for dosage adjustment based upon hepatic function, assessment of hepatic function is recommended before initiation of IXEMPRA and periodically thereafter.

### 8.7 Renal Impairment

Ixabepilone is minimally excreted via the kidney. No controlled pharmacokinetic studies were conducted with IXEMPRA in patients with renal impairment. IXEMPRA in combination with capecitabine has not been evaluated in patients with calculated creatinine clearance of <50 mL/min. IXEMPRA as monotherapy has not been evaluated in patients with creatinine >1.5 times ULN. In a population pharmacokinetic analysis of IXEMPRA as monotherapy, there was no meaningful effect of mild and moderate renal insufficiency (CrCL >30 mL/min) on the pharmacokinetics of ixabepilone.

## 10 OVERDOSAGE

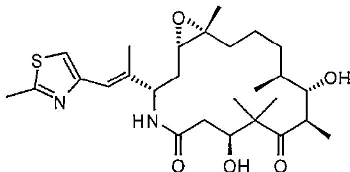
Experience with overdose of IXEMPRA is limited to isolated cases. The adverse reactions reported in these cases included peripheral neuropathy, fatigue, musculoskeletal pain/myalgia, and gastrointestinal symptoms (nausea, anorexia, diarrhea, abdominal pain, stomatitis). The highest dose mistakenly received was 100 mg/m$^2$ (total dose 185 mg).

There is no known antidote for overdosage of IXEMPRA. In case of overdosage, the patient should be closely monitored and supportive treatment should be administered. Management of overdose should include supportive medical interventions to treat the presenting clinical manifestations.

## 11 DESCRIPTION

IXEMPRA (ixabepilone) is a microtubule inhibitor belonging to a class of antineoplastic agents, the epothilones and their analogs. The epothilones are isolated from the myxobacterium *Sorangium cellulosum*. Ixabepilone is a semisynthetic analog of epothilone B, a 16-membered polyketide macrolide, with a chemically modified lactam substitution for the naturally existing lactone.

The chemical name for ixabepilone is (1*S*,3*S*,7*S*,10*R*,11*S*,12*S*,16*R*)-7,11-dihydroxy-8,8,10,12,16-pentamethyl-3-[(1*E*)-1-methyl-2-(2-methyl-4-thiazolyl)ethenyl]-17-oxa-4-azabicyclo[14.1.0] heptadecane-5,9-dione, and it has a molecular weight of 506.7. Ixabepilone has the following structural formula:



IXEMPRA (ixabepilone) for injection is intended for intravenous infusion only after constitution with the supplied DILUENT and after further dilution with a specified infusion fluid [*see Instructions for Preparation and IV Administration (2.4)*]. IXEMPRA (ixabepilone) for injection is supplied as a sterile, non-pyrogenic single-use vial providing 15 mg or 45 mg ixabepilone as a lyophilized white powder. The DILUENT for IXEMPRA is a sterile, non-pyrogenic solution of 52.8% (w/v) purified polyoxyethylated castor oil and 39.8% (w/v) dehydrated alcohol, USP. The IXEMPRA (ixabepilone) for injection and the DILUENT for IXEMPRA are co-packaged and supplied as IXEMPRA *Kit*.

## 12 CLINICAL PHARMACOLOGY

### 12.1 Mechanism of Action

Ixabepilone is a semi-synthetic analog of epothilone B. Ixabepilone binds directly to β-tubulin subunits on microtubules, leading to suppression of microtubule dynamics. Ixabepilone suppresses the dynamic instability of αβ-II and αβ-III microtubules. Ixabepilone possesses low *in vitro* susceptibility to multiple tumor resistance mechanisms including efflux transporters, such as MRP-1 and P-glycoprotein (P-gp). Ixabepilone blocks cells in the mitotic phase of the cell division cycle, leading to cell death.

### 12.2 Pharmacodynamics

In cancer patients, ixabepilone has a plasma concentration-dependent effect on tubulin dynamics in peripheral blood mononuclear cells that is observed as the formation of microtubule bundles. Ixabepilone has antitumor activity *in vivo* against multiple human tumor xenografts, including drug-resistant types that overexpress P-gp, MRP-1, and βIII tubulin isoforms, or harbor tubulin mutations. Ixabepilone is active in xenografts that are resistant to multiple agents including taxanes, anthracyclines, and vinca alkaloids. Ixabepilone demonstrated synergistic antitumor activity in combination with capecitabine *in vivo*. In addition to direct antitumor activity, ixabepilone has anti-angiogenic activity.

### 12.3 Pharmacokinetics

**Absorption**

Following administration of a single 40 mg/m$^2$ dose of IXEMPRA in patients with cancer, the mean $C_{max}$ was 252 ng/mL (coefficient of variation, CV 56%) and the mean AUC was 2143 ng•hr/mL (CV 48%). Typically $C_{max}$ occurred at the end of the 3 hour infusion. In cancer patients, the pharmacokinetics of ixabepilone were linear at doses of 15 to 57 mg/m$^2$.

**Distribution**

The mean volume of distribution of 40 mg/m$^2$ ixabepilone at steady-state was in excess of 1000 L. *In vitro*, the binding of ixabepilone to human serum proteins ranged from 67 to 77%, and the blood-to-plasma concentration ratios in human blood ranged from 0.65 to 0.85 over a concentration range of 50 to 5000 ng/mL.

**Metabolism**

Ixabepilone is extensively metabolized in the liver. *In vitro* studies indicated that the main route of oxidative metabolism of ixabepilone is via CYP3A4. More than 30 metabolites of ixabepilone are excreted into human urine and feces. No single metabolite accounted for more than 6% of the administered dose. The biotransformation products generated from ixabepilone by human liver microsomes were not active when tested for *in vitro* cytotoxicity against a human tumor cell line.

*In vitro* studies using human liver microsomes indicate that clinically relevant concentrations of ixabepilone do not inhibit CYP3A4, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, or CYP2D6. Ixabepilone does not induce the activity or the corresponding mRNA levels of CYP1A2, CYP2B6, CYP2C9, or CYP3A4 in cultured human hepatocytes at clinically relevant concentrations. Therefore, it is unlikely that ixabepilone will affect the plasma levels of drugs that are substrates of CYP enzymes.

**Elimination**

Ixabepilone is eliminated primarily as metabolized drug. After an intravenous $^{14}$[C]-ixabepilone dose to patients, approximately 86% of the dose was eliminated within 7 days in feces (65% of the dose) and in urine (21% of the dose). Unchanged ixabepilone accounted for approximately 1.6% and 5.6% of the dose in feces and urine, respectively. Ixabepilone has a terminal elimination half-life of approximately 52 hours. No accumulation in plasma is expected for ixabepilone administered every 3 weeks.

**Effects of Age, Gender, and Race**

Based upon a population pharmacokinetic analysis in 676 cancer patients, gender, race, and age do not have meaningful effects on the pharmacokinetics of ixabepilone.

## 13 NONCLINICAL TOXICOLOGY

### 13.1 Carcinogenesis, Mutagenesis, Impairment of Fertility

Carcinogenicity studies with ixabepilone have not been conducted. Ixabepilone did not induce mutations in the microbial mutagenesis (Ames) assay and was not clastogenic in an *in vitro* cytogenetic assay using primary human lymphocytes. Ixabepilone was clastogenic (induction of micronuclei) in the *in vivo* rat micronucleus assay at doses ≥0.625 mg/kg/day.

IXEMPRA® (ixabepilone)

There were no effects on male or female rat mating or fertility at doses up to 0.2 mg/kg/day in both males and females (approximately one-fifteenth the expected human clinical exposure based on AUC). The effect of ixabepilone on human fertility is unknown. However, when rats were given an IV infusion of ixabepilone during breeding and through the first 7 days of gestation, a significant increase in resorptions and pre- and post-implantation loss and a decrease in the number of corpora lutea was observed at 0.2 mg/kg/day. Testicular atrophy or degeneration was observed in 6-month rat and 9-month dog studies when ixabepilone was given every 21 days at intravenous doses of 6.7 mg/kg (40 mg/m$^2$) in rats (approximately 2.1 times the expected clinical exposure based on AUC) and 0.5 and 0.75 mg/kg (10 and 15 mg/m$^2$) in dogs (approximately 0.2 and 0.4 times the expected clinical exposure based on AUC).

### 13.2 Animal Toxicology

**Overdose**

In rats, single intravenous doses of ixabepilone from 60 to 180 mg/m$^2$ (mean AUC values ≥8156 ng•h/mL) were associated with mortality occurring between 5 and 14 days after dosing, and toxicity was principally manifested in the gastrointestinal, hematopoietic (bone-marrow), lymphatic, peripheral-nervous, and male-reproductive systems. In dogs, a single intravenous dose of 100 mg/m$^2$ (mean AUC value of 6925 ng•h/mL) was markedly toxic, inducing severe gastrointestinal toxicity and death 3 days after dosing.

## 14 CLINICAL STUDIES

**Combination Therapy**

In an open-label, multicenter, multinational, randomized trial of 752 patients with metastatic or locally advanced breast cancer, the efficacy and safety of IXEMPRA (40 mg/m$^2$ every 3 weeks) in combination with capecitabine (at 1000 mg/m$^2$ twice daily for 2 weeks followed by 1 week rest) were assessed in comparison with capecitabine as monotherapy (at 1250 mg/m$^2$ twice daily for 2 weeks followed by 1 week rest). Patients were previously treated with anthracyclines and taxanes. Patients were required to have demonstrated tumor progression or resistance to taxanes and anthracyclines as follows:

- tumor progression within 3 months of the last anthracycline dose in the metastatic setting or recurrence within 6 months in the adjuvant or neoadjuvant setting, and
- tumor progression within 4 months of the last taxane dose in the metastatic setting or recurrence within 12 months in the adjuvant or neoadjuvant setting.

For anthracyclines, patients who received a minimum cumulative dose of 240 mg/m$^2$ of doxorubicin or 360 mg/m$^2$ of epirubicin were also eligible.

Sixty-seven percent of patients were White, 23% were Asian, and 3% were Black. Both arms were evenly matched with regards to race, age (median 53 years), baseline performance status (Karnofsky 70-100%), and receipt of prior adjuvant or neo-adjuvant chemotherapy (75%). Tumors were ER-positive in 47% of patients, ER-negative in 43%, HER2-positive in 15%, HER2-negative in 61%, and ER-negative, PR-negative, HER2-negative in 25%. The baseline disease characteristics and previous therapies for all patients (n=752) are shown in Table 6.

**Table 6: Baseline Disease Characteristics and Previous Therapies**

| | IXEMPRA with capecitabine n=375 | Capecitabine n=377 |
|---|---|---|
| **Site of disease** | | |
| Visceral disease (liver or lung) | 316 (84%) | 315 (84%) |
| Liver | 245 (65%) | 228 (61%) |
| Lung | 180 (48%) | 174 (46%) |
| Lymph node | 250 (67%) | 249 (66%) |
| Bone | 168 (45%) | 162 (43%) |
| Skin/soft tissue | 60 (16%) | 62 (16%) |
| **Number of prior chemotherapy regimens in metastatic setting[a]** | | |
| 0 | 27 (7%) | 33 (9%) |
| 1 | 179 (48%) | 184 (49%) |
| 2 | 152 (41%) | 138 (37%) |
| ≥3 | 17 (5%) | 22 (6%) |
| **Anthracycline resistance[b]** | 164 (44%) | 165 (44%) |
| **Taxane Resistance[c]** | | |
| Neoadjuvant/adjuvant setting | 40 (11%) | 44 (12%) |
| Metastatic setting | 327 (87%) | 319 (85%) |

[a] For IXEMPRA plus capecitabine versus capecitabine only, prior treatment in the metastatic setting included cyclophosphamide (25% vs. 23%), fluorouracil (22% vs. 16%), vinorelbine (11% vs. 12%), gemcitabine (9% each arm), carboplatin (9% vs. 7%), liposomal doxorubicin (3% each arm), and cisplatin (2% vs. 3%).

[b] Tumor progression within 3 months in the metastatic setting or recurrence within 6 months in the adjuvant or neoadjuvant setting.

[c] 24% and 21% of patients had received 2 or more taxane-containing regimens in the combination and single agent treatment groups, respectively.

The patients in the combination treatment group received a median of 5 cycles of treatment and patients in the capecitabine monotherapy treatment group received a median of 4 cycles of treatment.

The primary endpoint of the study was progression-free survival (PFS) defined as time from randomization to radiologic progression as determined by Independent Radiologic Review (IRR), clinical progression of measurable skin lesions or death from any cause. Other study endpoints included objective tumor response based on Response Evaluation Criteria in Solid Tumors (RECIST), time to response, response duration, and overall survival.

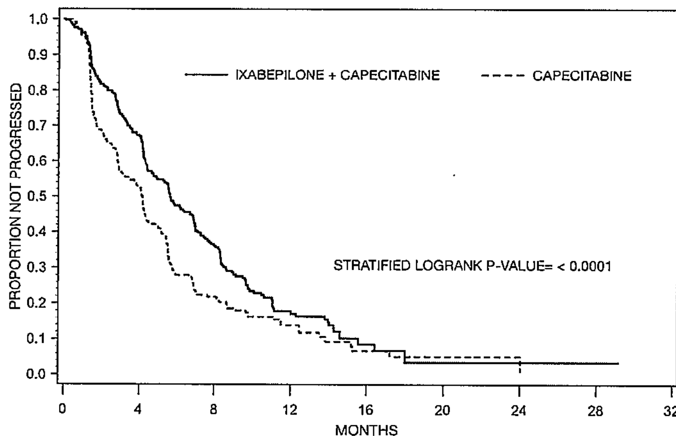
IXEMPRA in combination with capecitabine resulted in a statistically significant improvement in PFS compared to capecitabine. The results of the study are presented in Table 7 and Figure 1.

**Table 7: Efficacy of IXEMPRA in Combination with Capecitabine vs Capecitabine Alone – Intent-to-Treat Analysis**

| Efficacy Parameter | IXEMPRA with Capecitabine n=375 | Capecitabine n=377 |
|---|---|---|
| PFS | | |
| Number of events[a] | 242 | 256 |
| Median | 5.7 months | 4.1 months |
| (95% CI) | (4.8 - 6.7) | (3.1 - 4.3) |
| Hazard Ratio[b] (95% CI) | 0.69 (0.58 - 0.83) | |
| p-value[c] (Log rank) | <0.0001 | |
| Objective Tumor Response Rate (95% CI) | 34.7% (29.9 - 39.7) | 14.3% (10.9 - 18.3) |
| p-value[c] (CMH)[d] | <0.0001 | |
| Duration of Response, Median (95% CI) | 6.4 months (5.6 - 7.1) | 5.6 months (4.2 - 7.5) |

[a] Patients were censored for PFS at the last date of tumor assessment prior to the start of subsequent therapy. In patients where independent review was not available PFS was censored at the randomization date.
[b] For the hazard ratio, a value less than 1.00 favors combination treatment.
[c] Stratified by visceral metastasis in liver/lung, prior chemotherapy in metastatic setting, and anthracycline resistance.
[d] Cochran-Mantel-Haenszel test.

**Figure 1: Progression-free Survival Kaplan Meier Curves**




There was no statistically significant difference in overall survival between treatment arms in this study, as well as in a second similar study. In the study described above, the median overall survivals were 12.9 months (95% CI: 11.5, 14.2) in the combination therapy arm and 11.1 months (95% CI: 10.0, 12.5) in the capecitabine alone arm [Hazard Ratio 0.90 (95% CI: 0.77, 1.05), p-value=0.19].

In the second trial, comparing IXEMPRA in combination with capecitabine versus capecitabine alone, conducted in 1221 patients pretreated with an anthracycline and taxane, the median overall survivals were 16.4 months (95% CI: 15.0, 17.9) in the combination therapy arm and 15.6 months (95% CI: 13.9, 17.0), in the capecitabine alone arm [Hazard Ratio 0.90 (95% CI: 0.78, 1.03), p-value=0.12].

**Monotherapy**

IXEMPRA was evaluated as a single agent in a multicenter single-arm study in 126 women with metastatic or locally advanced breast cancer. The study enrolled patients whose tumors had recurred or had progressed following two or more chemotherapy regimens including an anthracycline, a taxane, and capecitabine. Patients who had received a minimum cumulative dose of 240 mg/m² of doxorubicin or 360 mg/m² of epirubicin were also eligible. Tumor progression or recurrence were prospectively defined as follows:

- Disease progression while on therapy in the metastatic setting (defined as progression while on treatment or within 8 weeks of last dose),
- Recurrence within 6 months of the last dose in the adjuvant or neoadjuvant setting (only for anthracycline and taxane),
- HER2-positive patients must also have progressed during or after discontinuation of trastuzumab.

In this study, the median age was 51 years (range, 30-78), and 79% were White, 5% Black, and 2% Asian, Karnofsky performance status was 70-100%, 88% had received two or more prior chemotherapy regimens for metastatic disease, and 86% had liver and/or lung metastases. Tumors were ER-positive in 48% of patients, ER-negative in 44%, HER2-positive in 7%, HER2-negative in 72%, and ER-negative, PR-negative, HER2-negative in 33%.

IXEMPRA was administered at a dose of 40 mg/m² intravenously over 3 hours every 3 weeks. Patients received a median of 4 cycles (range 1 to 18) of IXEMPRA therapy.

Objective tumor response was determined by independent radiologic and investigator review using RECIST. Efficacy results are presented in Table 8.

**Table 8: Efficacy of IXEMPRA in Metastatic and Locally Advanced Breast Cancer**

| Endpoint | Result |
|---|---|
| Objective tumor response rate (95% CI) | |
| IRR Assessment[a] (n=113) | 12.4% (6.9 - 19.9) |
| Investigator Assessment (n=126) | 18.3% (11.9 - 26.1) |
| Time to response[b] (n=14) | |
| Median, weeks (min - max) | 6.1 (5 - 54.4) |
| Duration of response[b] (n=14) | |
| Median, months (95% CI) | 6.0 (5.0 - 7.6) |

[a] All responses were partial.
[b] As assessed by IRR.

## 15 REFERENCES

1. Preventing Occupational Exposures to Antineoplastic and Other Hazardous Drugs in Health Care Settings. NIOSH Alert 2004-165.

2. OSHA Technical Manual, TED 1-0.15A, Section VI: Chapter 2. Controlling Occupational Exposure to Hazardous Drugs. OSHA, 1999. http://www.osha.gov/dts/osta/otm/otm_vi/otm_vi_2.html

3. American Society of Health-System Pharmacists. ASHP guidelines on handling hazardous drugs. *Am J Health-Syst Pharm.* 2006;63:1172-1193.

4. Polovich, M., White, J.M., & Kelleher, L.O. (eds.) 2005. Chemotherapy and biotherapy guidelines and recommendations for practice (2nd. ed.) Pittsburgh, PA: Oncology Nursing Society.

## 16 HOW SUPPLIED/STORAGE AND HANDLING

IXEMPRA is supplied as a *Kit* containing one vial of IXEMPRA® (ixabepilone) for injection and one vial of DILUENT for IXEMPRA.

| | |
|---|---|
| NDC 0015-1910-12 | IXEMPRA® *Kit* containing one vial of IXEMPRA® (ixabepilone) for injection, 15 mg and one vial of DILUENT for IXEMPRA, 8 mL |
| NDC 0015-1911-13 | IXEMPRA® *Kit* containing one vial of IXEMPRA® (ixabepilone) for injection, 45 mg and one vial of DILUENT for IXEMPRA, 23.5 mL |

IXEMPRA *Kit* must be stored in a refrigerator at 2° C to 8° C (36° F to 46° F). Retain in original package until time of use to protect from light.

Procedures for proper handling and disposal of antineoplastic drugs [*see References (15)*] should be followed. To minimize the risk of dermal exposure, impervious gloves should be worn when handling vials containing IXEMPRA, regardless of the setting, including unpacking and inspection, transport within a facility, and dose preparation and administration.

## 17 PATIENT COUNSELING INFORMATION

[*see FDA-Approved Patient Labeling (17.6)*]

### 17.1 Peripheral Neuropathy

Patients should be advised to report to their physician any numbness and tingling of the hands or feet [*see Warnings and Precautions (5.1)*].

### 17.2 Fever/Neutropenia

Patients should be instructed to call their physician if a fever of 100.5° F or greater or other evidence of potential infection such as chills, cough, or burning or pain on urination develops [*see Warnings and Precautions (5.2)*].

### 17.3 Hypersensitivity Reactions

Patients should be advised to call their physician if they experience urticaria, pruritus, rash, flushing, swelling, dyspnea, chest tightness or other hypersensitivity-related symptoms following an infusion of IXEMPRA [*see Warnings and Precautions (5.4)*].

### 17.4 Pregnancy

Patients should be advised to use effective contraceptive measures to prevent pregnancy and to avoid nursing during treatment with IXEMPRA [*see Warnings and Precautions (5.5) and Use in Specific Populations (8.1, 8.3)*].

### 17.5 Cardiac Adverse Reactions

Patients should be advised to report to their physician chest pain, difficulty breathing, palpitations or unusual weight gain [*see Warnings and Precautions (5.6)*].

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Exhibit 1029-83

## 17.6 FDA-Approved Patient Labeling

## Patient Information
## IXEMPRA® *Kit* (pronounced as ĭk-'sĕm-pră)
## (ixabepilone)
### for Injection, for intravenous infusion only

Read the Patient Information that comes with IXEMPRA before you start receiving it and before each injection. There may be new information. This leaflet does not take the place of talking with your healthcare provider about your medical condition or your treatment.

**What is the most important information I should know about IXEMPRA?**

Your healthcare provider should do blood tests to check your liver function:

- before you begin receiving IXEMPRA
- as needed while you are receiving IXEMPRA

If blood tests show that you have liver problems, do not receive injections of IXEMPRA along with the medicine capecitabine. Taking these two medicines together if you have liver problems increases your chance of serious problems. These include: serious infection and death due to a very low white blood cell count (neutropenia).

**What is IXEMPRA?**

IXEMPRA is a cancer medicine. IXEMPRA is used alone or with another cancer medicine called capecitabine. IXEMPRA is used to treat breast cancer, when certain other medicines have not worked or no longer work.

**Who should not take IXEMPRA?**

Do not receive injections of IXEMPRA if you:

- are allergic to a medicine, such as TAXOL®, that contains Cremophor® EL or polyoxyethylated castor oil.
- have low white blood cell or platelet counts. Your healthcare provider will check your blood counts.
- are also taking a cancer medicine called capecitabine and you have liver problems. **See "What is the most important information I should know about IXEMPRA?"**

**What should I tell my healthcare provider before receiving IXEMPRA?**

IXEMPRA may not be right for you. Before you receive IXEMPRA, tell your healthcare provider about all of your medical conditions, including if you:

- have liver problems
- have heart problems or a history of heart problems
- have had an allergic reaction to IXEMPRA. You will receive medicines before each injection of IXEMPRA to decrease the chance of an allergic reaction. **See "How will I receive IXEMPRA?"**
- are pregnant or plan to become pregnant. You should not receive IXEMPRA during pregnancy because it may harm your unborn baby. Talk with your healthcare provider about how to prevent pregnancy while receiving IXEMPRA. Tell your healthcare provider right away if you become pregnant or think you are pregnant while receiving IXEMPRA.
- are breast-feeding. It is not known if IXEMPRA passes into breast milk. You and your healthcare provider should decide if you will take IXEMPRA or breast-feed. You should not do both.

Tell your healthcare provider about all the medicines you take, including prescription and non-prescription medicines, vitamins, and herbal supplements.

IXEMPRA and certain other medicines may affect each other causing side effects. IXEMPRA may affect the way other medicines work, and other medicines may affect how IXEMPRA works. Know the medicines you take. Keep a list of your medicines with you to show your healthcare provider.

**How will I receive IXEMPRA?**

IXEMPRA is given by an injection directly into your vein (intravenous infusion). IXEMPRA is usually given once every three weeks. Each treatment with IXEMPRA will take about 3 hours.

Your healthcare provider will decide how much IXEMPRA you will receive and how often you will receive it.

To lower the chance of allergic reaction, you will receive other medicines about 1 hour before each treatment with IXEMPRA. **(See "What are the possible side effects of IXEMPRA?")**

If you have an allergic reaction to IXEMPRA, you will receive a steroid medicine before future doses of IXEMPRA. You may also need to receive your doses of IXEMPRA more slowly.

**What should I avoid while receiving IXEMPRA?**

IXEMPRA contains alcohol. If you are dizzy or drowsy, avoid activities that may be dangerous, such as driving or operating machinery.

Do not drink grapefruit juice while receiving IXEMPRA. Drinking grapefruit juice may cause you to have too much IXEMPRA in your blood and lead to side effects.

**What are the possible side effects of IXEMPRA?**

IXEMPRA may cause serious side effects including:

- **Numbness, tingling, or burning in the hands or feet can occur while taking IXEMPRA (neuropathy).** These symptoms may be new or get worse while you are receiving IXEMPRA. These symptoms often occur early during treatment with IXEMPRA. Tell your healthcare provider if you have any of these symptoms. Your dose of IXEMPRA may need to be decreased, stopped until your symptoms get better, or totally stopped.

IXEMPRA® (ixabepilone)

- **Low white blood cell count (neutropenia).** White blood cells help protect the body from infections caused by bacteria. If you get a fever or infection when your white blood cells are very low, you can become seriously ill and die. You may need treatment in the hospital with antibiotic medicines. Your healthcare provider will monitor your white blood cell count often with blood tests. Tell your healthcare provider right away or go to the nearest hospital emergency room if you have a fever (temperature above 100.5° F) or other sign of infection, such as chills, cough, burning or pain when you urinate, any time between treatments with IXEMPRA.
- **Allergic Reactions.** Severe allergic reactions can occur with IXEMPRA and may cause death in rare cases. Allergic reactions are most likely to occur while IXEMPRA is being injected into your vein. Tell your healthcare provider right away if you get any of the following signs and symptoms of an allergic reaction:
  - itching, hives (raised itchy welts), rash
  - flushed face
  - sudden swelling of face, throat or tongue
  - chest tightness, trouble breathing
  - feel dizzy or faint
  - feel your heart beating (palpitations)
  - **Harm to an unborn child. See "What should I tell my healthcare provider before receiving IXEMPRA?"**
  - **Heart problems.** IXEMPRA might cause decreased blood flow to the heart, problems with heart function, and abnormal heart beat. This is seen more often in patients who also take capecitabine. **Tell your healthcare provider right away if you have any of the following symptoms:**
    - chest pain,
    - difficulty breathing,
    - feel your heart beating (palpitations), or
    - unusual weight gain.

The most common side effects with IXEMPRA used alone or with capecitabine may include:

- tiredness
- loss of appetite
- disorders of toenails and fingernails
- hair loss
- fever
- decreased red blood cells (anemia)
- joint and muscle pain
- headache
- decreased platelets (thrombocytopenia)
- nausea, vomiting, diarrhea, constipation, and abdominal pain
- sores on the lip, in the mouth and esophagus
- tender, red palms and soles of feet (hand-foot syndrome) that looks like a sunburn; the skin may become dry and peel. There may also be numbness and tingling.

Tell your healthcare provider about any side effect that bothers you or that does not go away.

These are not all of the side effects of IXEMPRA. Ask your healthcare provider or pharmacist for more information if you have questions or concerns.

**General information about IXEMPRA**

This patient information leaflet summarizes the most important information about IXEMPRA. Medicines are sometimes prescribed for purposes other than those listed in a Patient Information Leaflet. If you would like more information about IXEMPRA, talk with your healthcare provider. You can ask your healthcare provider or pharmacist for information about IXEMPRA that is written for health professionals. For more information about IXEMPRA, call 1-888-IXEMPRA.

IXEMPRA® (ixabepilone) for injection Manufactured by: Baxter Oncology GmbH,
33790 Halle/Westfalen, Germany

DILUENT for IXEMPRA Manufactured by: Baxter Oncology GmbH,
33790 Halle/Westfalen, Germany

Distributed by Bristol-Myers Squibb Company, Princeton, NJ 08543 USA

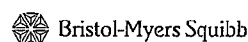


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Exhibit 1029-84

# EXHIBIT I

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Article Link: http://www.webmd.com/breast-cancer/news/20091215/targeted-breast-cancer-drug-shrinks-tumors

## Breast Cancer Health Center

# Targeted Breast Cancer Drug Shrinks Tumors

### Study Shows T-DM1 Helps Patients Who Were Unsuccessfully Treated With Other Drugs

By Charlene Laino
WebMD Health News
Reviewed by Louise Chang, MD

Dec. 15, 2009 (San Antonio) -- A new targeted cancer drug has been shown to shrink tumors in women with metastatic breast cancer after an average of seven other drugs, including Herceptin, failed.

The new drug, called T-DM1, combines Herceptin with a potent chemotherapy drug. It's a Trojan horse approach, where Herceptin homes in on cancer cells and delivers the cancer-killing agent directly to its target.

Tumors shrank in one-third of women with metastatic breast cancer given T-DM1, says Ian Krop, MD, of the Dana-Farber Cancer Institute in Boston. In another 12%, tumors stopped growing for at least six months.

The women remained cancer-free for an average of seven months -- results unheard of in patients this sick, he says.

All the women, who had breast tumors for an average of three years, had cancer that had metastasized, or spread to other parts of the body. They had been treated with an average of seven different therapies, including Herceptin, Tykerb, and Xeloda, and each had failed.

"This is the first study looking at women who have failed so many other treatments," Krop tells WebMD. "But we think these results are as good as we've ever seen is such a refractory [sick] population," he says.

The findings were presented at the San Antonio Breast Cancer Symposium.

✓ Get Your Personalized Breast Cancer Treatment Report

**How T-DM1 Works**

About 20% of breast cancer patients have HER2-positive cancers -- tumors that have too much of a type of protein called HER2. Herceptin, a man-made antibody, binds to and blocks the HER2 receptor that appears on the surface of some breast cancer cells.

But metastatic breast cancer eventually becomes resistant to Herceptin. So researchers have searched for new drugs that target HER2.

T-DM1 is such a drug. The "T" stands for trastuzumab, the scientific name for Herceptin. The "DM1" is derived from an old chemotherapy drug called maytansine that was abandoned several decades ago when it was found to be too toxic for patients, Krop says.

Because Herceptin only zeroes in on cancer cells that express HER2, DM1 is delivered only to those cells, he says.

"The cytotoxic drug goes right to the cancer cells, so it's not floating around and causing other problems. And Herceptin still does all the things that Herceptin does" to fight cancer, Krop says.

All the women experienced some side effects, typically the fatigue and nausea often seen with chemotherapy, he says.

Edith Perez, MD, a breast cancer specialist at the Mayo Clinic in Jacksonville, Fla., tells WebMD that the drug's benefits far outweigh the risks.

"The response rate they saw in the study is exceptional in a group of patients this ill," she says.

One patient with pre-existing fatty liver disease and multiple other medical conditions died from liver failure.

Perez says that the death due to liver failure is not overly concerning given the women's overall poor health."These patients are very sick and right now we have no choices to offer them."

In other ongoing studies, T-DM1 is being pitted against other cancer drugs in patients with both metastatic and earlier-stage breast cancer, Krop says. Researchers expect T-DM1 will perform even better in women with earlier-stage cancer, he adds.

The study was funded by Genentech and Hoffmann-La Roche, which are developing the new drug.

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# EXHIBIT J

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# Roche-ImmunoGen to Start T-DM1 Trials

**Adam Feuerstein**

12/14/09 - 12:15 AM EST

WALTHAM, Mass. (TheStreet) -- **ImmunoGen**(IMGN Quote) is expected to announce Monday a commitment by its partner **Roche** to begin new late-stage clinical trials of the experimental breast cancer drug T-DM1.

The overall goal of this new clinical trial program will be to determine whether T-DM1, which is still not approved for use, can one day replace Roche's Herceptin as the leading treatment for women with certain breast cancers.

Herceptin global sales totaled $3.8 billion in the first nine months of 2009 and $4.7 billion in sales in 2008, according to Roche.

The **Genentech** unit of Roche will start a phase III study of T-DM1 in first-line breast cancer in May 2010. Additionally, a clinical trial designed to evaluate T-DM1 in the post-surgical, or adjuvant, setting is also in the planning stages, Immunogen will announce.

T-DM1 is a second-generation version of Roche's Herceptin. It consists of Herceptin (also known as trastuzumab) linked to a tumor-killing chemotherapy payload developed by ImmunoGen.

Roche's new commitment to advance T-DM1 comes as the drug maker plans to meet with the U.S. Food and Drug Administration to discuss seeking approval for T-DM1 as a third-line treatment for breast cancer patients.

On Saturday, researchers presented new clinical data from a phase II study showing that T-DM1 shrank tumors in 33% of critically ill breast cancer patients. The 110 patients in this study were essentially out of medical options because their breast cancer was no longer responding to treatment with Herceptin or **GlaxoSmithKline's**(GSK Quote) Tykerb.

Patients in the study also went an average of 7.3 months before their tumors began to grow again.

"These are breast cancer patients who do not have a lot options at this point. And what we see with T-DM1 is that it really offers benefit. I'm quite encouraged and I think this drug is significant for patients who really need help," said Dr. Ian Krop of the Boston's Dana Farber Cancer Institute Krop and the lead investigator of the T-DM1 study.

Based on these data, Krop said he'd like to see Roche seek regulatory approval soon.

"T-DM1 is a drug that I'd like to see made available to patients. I'm sure Genentech [Roche] is strongly considering using this data for the basis of an accelerated approval filing," he said.

Krop presented his findings from the T-DM1 study at the San Antonio Breast Cancer Symposium. ImmunoGen first disclosed partial results from the study on Wednesday.

T-DM1, like Herceptin, is designed to be most effective in patients with excess levels of a protein known as Her-2 that causes breast tumors to grow more aggressively. Almost 40% of patients with confirmed Her-2 positive status in the study saw their breast cancer tumors shrink.

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Exhibit 1029-89

If Roche does seek FDA approval for T-DM1, the drug would be the first treatment approved as a third-line treatment for breast cancer. T-DM1 would also be the first approved drug for ImmunoGen, which is set to receive mid single-digit royalties on T-DM1 sales by Roche.

The T-DM1 data presented Saturday "are quite the validating statement for our technology," said ImmunoGen CEO Dan Junius. "A year from now, we could have T-DM1 on the market for an indication where there is no approved therapy today."

Roche is conducting already conducting a separate phase III study testing T-DM1 head-to-head against Glaxo's Tykerb in second-line breast cancer patients. A phase II study of T-DM1 compared to Herceptin in first-line breast cancer will have data ready for presentation at an upcoming medical meeting.

ImmunoGen shares closed Friday at $8.77.

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Exhibit 1029-90

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IDC calculates the Market Cap for the basic symbol to include common shares only. Year-to-date mutual fund returns are calculated on a monthly basis by Value Line and posted mid-month.

*Oil Data in Market Overview is Brent Crude Pricing



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