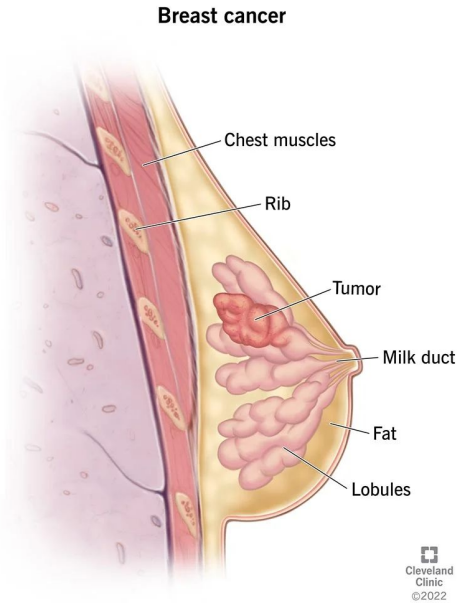
Survival Analysis: Time Until Death of Breast Cancer Patients

Hannah Chiu

Breast Cancer and Study Background

Breast Cancer

- Current prevalence in US: 129.7/100,000 women[1]
- **Primary invasive breast cancer**: there is/are **malignant tumor(s) that originate in the ducts or lobules** and have spread to surrounding tissues, but not other organs



Data Source

- Observational study
- Tumor tissue samples taken from three hospitals in Rotterdam, Netherlands[3]
  - St. Clara Hospital
  - Ikazia Hospital
  - St. Fransiscus Gasthuis Hospital
- 2780 pre- and postmenopausal cisgender women with primary, operable, invasive breast cancer

[CANCER RESEARCH 60, 636–643, February 1, 2000]

The Urokinase System of Plasminogen Activation and Prognosis in 2780 Breast Cancer Patients[1]

John A. Foekens,[2] Harry A. Peters, Maxime P. Look, Henk Portengen, Manfred Schmitt, Michael D. Kramer, Nils Brünner, Fritz Jänicke, Marion E. Meijer-van Gelder, Sonja C. Henzen-Logmans, Wim L. J. van Putten, and Jan G. M. Klijn

Division of Endocrine Oncology, Department of Medical Oncology [J. A. F., H. A. P., M. P. L., H. P., M. E. M-v. G., J. G. M. K.], and Departments of Statistics [W. L. J. v. P.] and Pathology [S. C. H-L.], Rotterdam Cancer Institute (Daniel den Hoed Kliniek)/Academic Hospital, NL-3075 Rotterdam, the Netherlands; the Frauenklinik der Technischen Universität München, Klinikum rechts der Isar, D-81675 München, Germany [M. S.]; Institut für Immunologie und Serologie der Universität, D-69120 Heidelberg, Germany [M. D. K.]; Finsen Laboratory, Rigshospitalet, DK-2100 Copenhagen, Denmark [N. B.]; and Frauenklinik und Poliklinik, Universitäts-Krankenhaus der Universität Hamburg, D-20246 Hamburg, Germany [F. J.]

ABSTRACT

The antigen levels of components of the urokinase-type plasminogen activator (uPA) system of plasminogen activation are correlated with prognosis in several types of cancers, including breast cancer. In the present study involving 2780 patients with primary invasive breast cancer, we have evaluated the prognostic importance of the four major components of the uPA system [uPA, the receptor uPAR (CD87), and the inhibitors PAI-1 and PAI-2]. The antigen levels were determined by ELISA in cytosols prepared from primary breast tumors. The levels of the four factors significantly correlated with each other; the Spearman rank correlation coefficients (r_s) ranged from 0.32 (between PAI-2 and PAI-1 or uPAR) to 0.59 (between uPA and PAI-1). The median duration of follow-up of patients still alive was 88 months. In the multivariate analyses for relapse-free survival (RFS) and overall survival (OS), we defined a basic model including age, menopausal status, tumor size and grade, lymph node status, adjuvant therapy, and steroid hormone receptor status. uPA, uPAR, PAI-1, and PAI-2 were considered as categorical variables, each with two cut points that were established by isotonic regression analysis. Compared with tumors with low levels, those with intermediate and high levels showed a relative hazard rate (RHR) and 95% confidence interval (95% CI) of 1.22 (1.02–1.45) and 1.69 (1.39–2.05) for uPA, and 1.32 (1.14–1.54) and 2.17 (1.74–2.70) for PAI-1, respectively, in multivariate analysis for RFS in all patients. Compared with tumors with high PAI-2 levels, those with intermediate and low levels showed a poor RFS with a RHR (95% CI) of 1.30 (1.14–1.48) and 1.76 (1.38–2.24), respectively. Similar results were obtained in the multivariate analysis for OS in all patients. Furthermore, uPA and PAI-1 were independent predictive factors of a poor RFS and OS in node-negative and node-positive patients. PAI-2 also added to the multivariate models for RFS in node-negative and node-positive patients, and in the analysis for OS in node-negative patients. uPAR did not further contribute to any of the multivariate models. A prognostic score was calculated based on the estimates from the final multivariate model for RFS. Using this score, the difference between the highest and lowest 10% risk groups was 66% in the analysis for RFS at 10 years and 61% in the analysis for OS. Moreover, separate prognostic scores were calculated for node-negative and node-positive patients. In the 10% highest risk groups, the proportion of disease-free patients was only 27 ± 6% and 9 ± 3% at 10 years for node-negative and node-positive patients, respectively. These proportions were 86 ± 4% and 61 ± 6% for the corresponding 10% lowest risk groups of relapse. We conclude that several components of the uPA system are potential predictors of RFS and OS in patients with primary invasive breast cancer. Knowledge of these factors could be helpful to assess the individual risk of patients, to select various types of adjuvant treatment and to identify patients who may benefit from targeted therapies that are currently being developed.

INTRODUCTION

Cancer cell invasion and metastasis result from a coordinated interaction between proteolytic enzymes degrading the ECM[3] and the adhesive proteins playing a role in cell attachment and migration. Data from preclinical and clinical studies point toward a central role for the uPA system in these processes (reviewed in Refs. 1–4). The serine protease uPA, which binds to a specific cell surface receptor uPAR (5, 6), facilitates the conversion of plasminogen into the serine protease plasmin. This wide-spectrum protease is able to degrade most components of the ECM directly or indirectly through activation of metalloproteinases, which subsequently degrade collagens and other matrix proteins (reviewed in Ref. 7). The activity of uPA can be inhibited by the serpin inhibitors PAI-1 and PAI-2 (8). In addition, most components of the uPA system of plasminogen activation have been linked to cell adhesion and migration through both proteolytic and nonproteolytic mechanisms (reviewed in Refs. 1–3). Cell migration requires the interaction of cell-bound adhesion receptors, such as integrins and uPAR, with their ECM-associated ligands such as vitronectin (9–12). Binding of uPA, or fragments of uPA containing only the receptor binding domain, enhance binding of uPAR to vitronectin. PAI-1 can inhibit integrin and uPAR binding to vitronectin, thus directing a stepwise cell migration by allowing tumor cells to be attached or alternatively being detached from the ECM (10, 11, 13, 14).

Duffy *et al*. (15) were the first to link increased levels of uPA activity in breast tumor extracts with a high rate of relapse in patients with breast cancer. This important finding of an association between uPA and a poor prognosis has been confirmed by various research groups measuring uPA antigen levels (16) in breast tumors, as well as in a variety of other cancer types (reviewed in Refs. 4, 17). Interestingly, immunocytologically detected uPA-positive tumor cells in bone marrow from primary breast cancer patients were predictive of a poor prognosis (18). Moreover, as reported by Jänicke *et al*. (19), surprisingly at first, increased levels of the inhibitor PAI-1 were associated with a poor prognosis in primary breast cancer (4, 17), and like uPA, also in recurrent breast cancer treated with tamoxifen (20). These findings can now partly be explained by the recently ascribed role of PAI-1 in tumor cell adhesion and migration (1–3). As would be expected, high tumor levels of uPAR were associated with a poor prognosis (21, 22), and high levels of PAI-2 were associated with a favorable prognosis in patients with breast cancer (23, 24).

Because simultaneous measurement of the different components of the uPA system of plasminogen activation may provide more powerful prognostic information, we determined the levels of uPA, uPAR,

Received 7/8/99; accepted 12/2/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[1] Supported by Grant DDHK 96-1234 of the Dutch Cancer Society, Amsterdam, the Netherlands.

[2] To whom requests for reprints should be addressed, at Josephine Nefkens Institute, Dr. Molewaterplein 50, Room Be426, 3015 GE Rotterdam, the Netherlands. Phone: (31)-10-4088 369; Fax: (31)-10-4088-377/365; E-mail: foekens@bidh.azr.nl.

[3] The abbreviations used are: ECM, extracellular matrix; uPA, urokinase-type plasminogen activator; uPAR, uPA receptor; PAI, plasminogen activator inhibitor; RFS, relapse-free survival; OS, overall survival; RHR, relative hazard rates; CI, confidence interval; df, degrees of freedom; $\Delta\chi^2$, increase in χ^2; CV, coefficient of variation; ER, estrogen receptor; PgR, progesterone receptor; IRA, isotonic regression analysis.

Data Source

- All patients examined routinely every 3-6 months during first five years of follow-up
  - Examined once a year after first five years
- Measured time until relapse of disease and/or death
- 1084 (39%) patients were lost to follow-up

[CANCER RESEARCH 60, 636–643, February 1, 2000]

The Urokinase System of Plasminogen Activation and Prognosis in 2780 Breast Cancer Patients[1]

John A. Foekens,[2] Harry A. Peters, Maxime P. Look, Henk Portengen, Manfred Schmitt, Michael D. Kramer, Nils Brünner, Fritz Jänicke, Marion E. Meijer-van Gelder, Sonja C. Henzen-Logmans, Wim L. J. van Putten, and Jan G. M. Klijn

Division of Endocrine Oncology, Department of Medical Oncology [J. A. F., H. A. P., M. P. L., H. P., M. E. M-v. G., J. G. M. K.], and Departments of Statistics [W. L. J. v. P.] and Pathology [S. C. H-L.], Interfaculty Cancer Institute (Daniel den Hoed Kliniek)/Academic Hospital, NL-3075 Rotterdam, the Netherlands; the Frauenklinik der Technischen Universität München, Klinikum rechts der Isar, D-81675 München, Germany [M. S.]; Institut für Immunologie und Serologie der Universität, D-69120 Heidelberg, Germany [M. D. K.]; Finsen Laboratory, Rigshospitalet, DK-2100 Copenhagen, Denmark [N. B.]; and Frauenklinik und Poliklinik, Universitäts-Krankenhaus der Universität Hamburg, D-20246 Hamburg, Germany [F. J.]

ABSTRACT

The antigen levels of components of the urokinase-type plasminogen activator (uPA) system of plasminogen activation are correlated with prognosis in several types of cancers, including breast cancer. In the present study involving 2780 patients with primary invasive breast cancer, we have evaluated the prognostic importance of the four major components of the uPA system [uPA, the receptor uPAR (CD87), and the inhibitors PAI-1 and PAI-2]. The antigen levels were determined by ELISA in cytosols prepared from primary breast tumors. The levels of the four factors significantly correlated with each other; the Spearman rank correlation coefficients (r_s) ranged from 0.32 (between PAI-2 and PAI-1 or uPAR) to 0.59 (between uPA and PAI-1). The median duration of follow-up of patients still alive was 88 months. In the multivariate analyses for relapse-free survival (RFS) and overall survival (OS), we defined a basic model including age, menopausal status, tumor size and grade, lymph node status, adjuvant therapy, and steroid hormone receptor status. uPA, uPAR, PAI-1, and PAI-2 were considered as categorical variables, each with two cut points that were established by isotonic regression analysis. Compared with tumors with low levels, those with intermediate and high levels showed a relative hazard rate (RHR) and 95% confidence interval (95% CI) of 1.22 (1.02–1.45) and 1.69 (1.39–2.05) for uPA, and 1.32 (1.14–1.54) and 2.17 (1.74–2.70) for PAI-1, respectively, in multivariate analysis for RFS in all patients. Compared with tumors with high PAI-2 levels, those with intermediate and low levels showed a poor RFS with a RHR (95% CI) of 1.30 (1.14–1.48) and 1.76 (1.38–2.24), respectively. Similar results were obtained in the multivariate analysis for OS in all patients. Furthermore, uPA and PAI-1 were independent predictive factors of a poor RFS and OS in node-negative and node-positive patients. PAI-2 also added to the multivariate models for RFS in node-negative and node-positive patients, and in the analysis for OS in node-negative patients. uPAR did not further contribute to any of the multivariate models. A prognostic score was calculated based on the estimates from the final multivariate model for RFS. Using this score, the difference between the highest and lowest 10% risk groups was 66% in the analysis for RFS at 10 years and 61% in the analysis for OS. Moreover, separate prognostic scores were calculated for node-negative and node-positive patients. In the 10% highest risk groups, the proportion of disease-free patients was only 27 ± 6% and 9 ± 3% at 10 years for node-negative and node-positive patients, respectively. These proportions were 86 ± 4% and 61 ± 6% for the corresponding 10% lowest risk groups of relapse. We conclude that several components of the uPA system are potential predictors of RFS and OS in patients with primary invasive breast cancer. Knowledge of these factors could be helpful to assess the individual risk of patients, to select various types of adjuvant treatment and to identify patients who may benefit from targeted therapies that are currently being developed.

Received 7/8/99; accepted 12/2/99.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked *advertisement* in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

[2] Supported by Grant DDHK 96-1234 of the Dutch Cancer Society, Amsterdam, the Netherlands.

[2] To whom requests for reprints should be addressed, at Josephine Nefkens Institute, Dr. Molewaterplein 50, Room Bo426, 3015 GE Rotterdam, the Netherlands. Phone: (31)-10-4088 369; Fax: (31)-10-4088-377/565; E-mail: foekens@bidh.azr.nl.

INTRODUCTION

Cancer cell invasion and metastasis result from a coordinated interaction between proteolytic enzymes degrading the ECM[3] and the adhesive proteins playing a role in cell attachment and migration. Data from preclinical and clinical studies point toward a central role for the uPA system in these processes (reviewed in Refs. 1–4). The serine protease uPA, which binds to a specific cell surface receptor uPAR (5, 6), facilitates the conversion of plasminogen into the serine protease plasmin. This wide-spectrum protease is able to degrade most components of the ECM directly or indirectly through activation of metalloproteinases, which subsequently degrade collagens and other matrix proteins (reviewed in Ref. 7). The activity of uPA can be inhibited by the serpin inhibitors PAI-1 and PAI-2 (8). In addition, most components of the uPA system of plasminogen activation have been linked to cell adhesion and migration through both proteolytic and nonproteolytic mechanisms (reviewed in Refs. 1–3). Cell migration requires the interaction of cell-bound adhesion receptors, such as integrins and uPAR, with their ECM-associated ligands such as vitronectin (9–12). Binding of uPA, or fragments of uPA containing only the receptor binding domain, enhance binding of uPAR to vitronectin. PAI-1 can inhibit integrin and uPAR binding to vitronectin, thus directing a stepwise cell migration by allowing tumor cells to be attached or alternatively being detached from the ECM (10, 11, 13, 14).

Duffy *et al.* (15) were the first to link increased levels of uPA activity in breast tumor extracts with a high rate of relapse in patients with breast cancer. This important finding of an association between uPA and a poor prognosis has been confirmed by various research groups measuring uPA antigen levels (16) in breast tumors, as well as in a variety of other cancer types (reviewed in Refs. 4, 17). Interestingly, immunocytologically detected uPA-positive tumor cells in bone marrow from primary breast cancer patients were predictive of a poor prognosis (18). Moreover, as reported by Jänicke *et al.* (19), surprisingly at first, increased levels of the inhibitor PAI-1 were associated with a poor prognosis in primary breast cancer (4, 17), and like uPA, also in recurrent breast cancer treated with tamoxifen (20). These findings can now partly be explained by the recently ascribed role of PAI-1 in tumor cell adhesion and migration (1–3). As would be expected, high tumor levels of uPAR were associated with a poor prognosis (21, 22), and high levels of PAI-2 were associated with a favorable prognosis in patients with breast cancer (23, 24).

Because simultaneous measurement of the different components of the uPA system of plasminogen activation may provide more powerful prognostic information, we determined the levels of uPA, uPAR,

[3] The abbreviations used are: ECM, extracellular matrix; uPA, urokinase-type plasminogen activator; uPAR, uPA receptor; PAI, plasminogen activator inhibitor; RFS, relapse-free survival; OS, overall survival; RHR, relative hazard rates; CI, confidence interval; df, degrees of freedom; $\Delta\chi^2$, increase in χ^2; CV, coefficient of variation; ER, estrogen receptor; PgR, progesterone receptor; IRA, isotonic regression analysis.

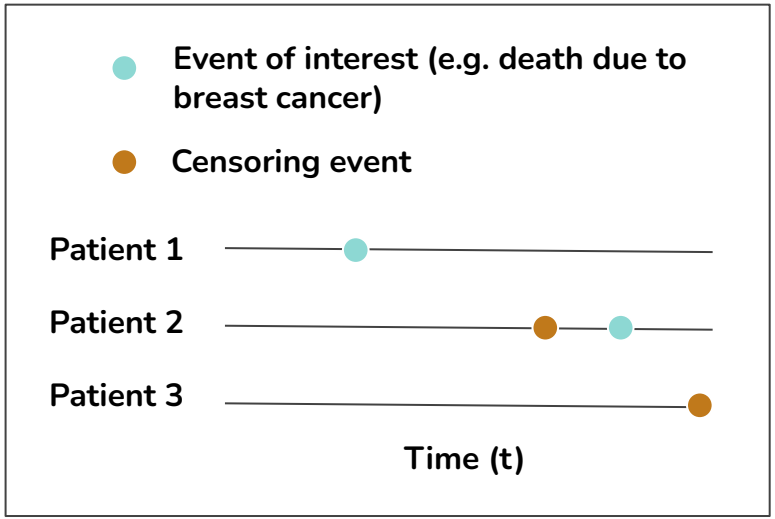
Is there a difference in survival time when undergoing chemotherapy vs. hormone therapy?

Modelling Survival

- **Survival analysis**: collection of statistical procedures for the analysis of data in which the outcome variable of interest is time until an event occurs
  - Graphically represented by Kaplan-Meier (KM) curves
  - KM curves are non-parametric

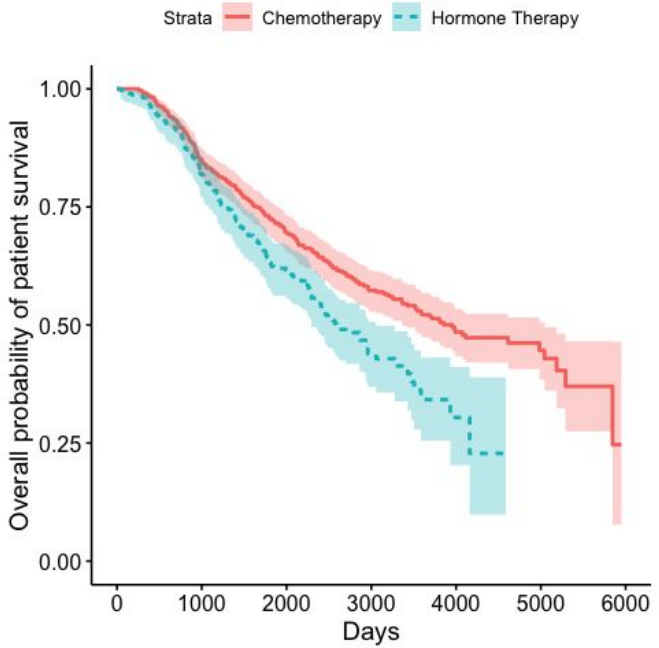
Censoring

- **Censoring**: occurs when we have information about individual survival time, but don't know the exact survival time
  - Right censoring is the most common
- Our data is right censored



Breast Cancer Patient Survival

- Only included patients who either received chemotherapy **or** hormone therapy
  - 552 patients received chemotherapy
  - 311 patients received hormone therapy



Log-rank Test

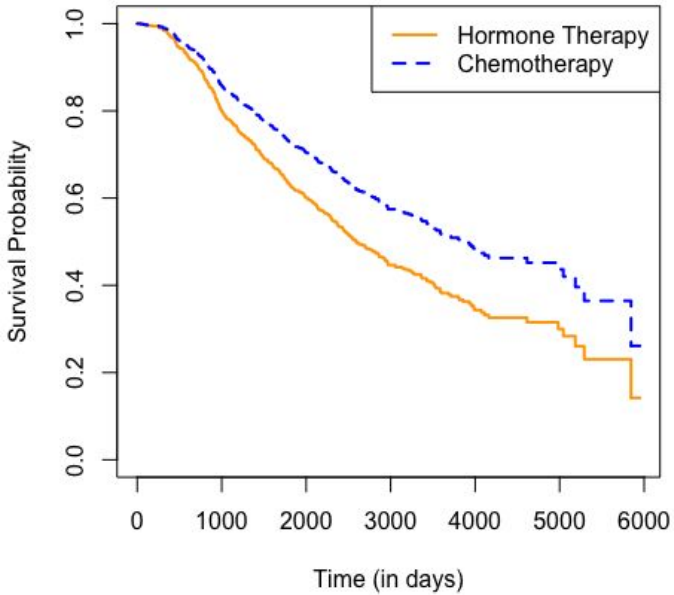
- **Log-rank test**: compares KM curves (is there a significant difference in the time until an event occurs?)
- Used log-rank test on KM curves from previous slide
    - Using a significance level of 0.05, I found significant evidence (**p = 3e-4**) that there is an association between the type of treatment received and survival time.

Cox Proportional Hazards (PH) Model

- **Cox PH model**: can evaluate (simultaneously) the effect of several factors on survival time
    - Semi-parametric model
    - Proportional hazards (PH) assumption: assumes hazard ratio is constant over time

Cox Proportional Hazards (PH) Model

- Hazard ratio is **1.45** (comparing hormone to chemo)
- 95% confidence interval: **1.18 - 1.78**
- Using a significance level of 0.05, I found significant evidence (**p = 4e-4**) that there is an association between type of treatment and survival time.



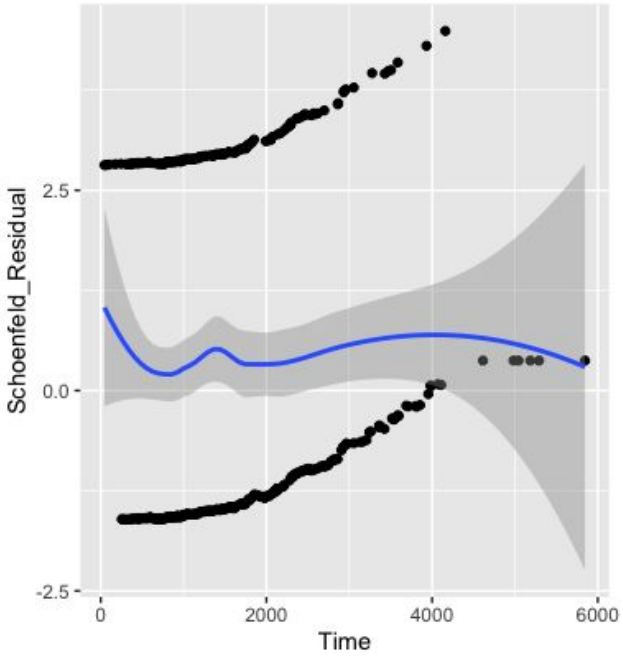
Adjusting for Other Factors

- When adjusting for number of positive lymph nodes and concentrations of estrogen and progesterone receptors:
  - Hazard ratio is **1.23** (comparing hormone to chemo)
  - 95% confidence interval: **0.99 - 1.53**
  - Using a significance level of 0.05, I found significant evidence (**p = 2e-16**) that there is an association between type of treatment and survival time when adjusting for other factors.

Diagnostics

Schoenfeld Residuals

- **Schoenfeld residuals**: used to test assumption of proportional hazards
  - A trend that isn't flat about 0 can be a sign of non-proportional hazards



Conclusions

Limitations

- This dataset comes from an observational study
- Small sample size
- Location and time that data was taken from

Final Thoughts

- There is an association between type of treatment received and survival
- Increased hazard for group treated with hormone therapy compared to chemotherapy
- There is a lot to consider when working with survival data in health!
    - Parametric, semi-parametric, vs. nonparametric
    - Censoring

References

1. U.S. Cancer Statistics Working Group. U.S. Cancer Statistics Data Visualizations Tool, based on 2021 submission data (1999-2019): U.S. Department of Health and Human Services, Centers for Disease Control and Prevention and National Cancer Institute; https://www.cdc.gov/cancer/dataviz, released in November 2022.
2. Centers for Disease Control and Prevention. (2022, September 26). *What are the symptoms of breast cancer?* Centers for Disease Control and Prevention. Retrieved February 22, 2023, from https://www.cdc.gov/cancer/breast/basic_info/symptoms.htm
3. Foekens, J., Peters, H., Look, M., Portengen, H., Schmitt, M., Kramer, M., Brünner, N., Jänicke, F., Meijer - Gelder, M., Henzen - Logmans, S., Putten, W., & Klijn, J. (2000). The urokinase system of plasminogen activation and prognosis in 2780 breast cancer patients. *Cancer Research (Chicago, Ill.)*, *60*(3), 636–643.